

## Anti-Staphylococcal activity of Aqueous Garlic Extract (AGE) Determined by Disc Diffusion Method

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An interventional study was conducted in Department of Pharmacology and Therapeutics in collaboration with Department of Microbiology, Mymensingh Medical College, Mymensingh to determine the antibacterial effect of aqueous extract of garlic (*Allium sativum*) against standard strain of *Staphylococcus aureus* ATCC 25923. Antibacterial effect of AGE was determined by disc diffusion method. Sensitivity of AGE determined in disc diffusion and the zone of inhibition (ZOI) was 8 mm, 14 mm and 22 mm at 25 µg/ 10 µl, 50 µg/ 10 µl and 100 µg/ 10 µl concentrations respectively. From the findings it is clearly determined that the extract has definite antibacterial effect upon *Staphylococcus aureus*. Further studies are required to detect and isolate the active ingredients present in the Garlic extract as well as detail steps of mechanism responsible for antibacterial effect. Then their effects against the studied organism should be studied in vivo separately and its toxicity profile should also be taken into account. Only then the Garlic extract fulfilled the criteria for its therapeutic use.

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**Key words:** Antibacterial effect, Aqueous Garlic Extract, *Staphylococcus aureus*, Zone of Inhibition (ZOI)

### Introduction

Different extracts from traditional medicinal plants were tasted and some natural products were approved as new antibacterial drugs. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance.<sup>1-3</sup> Garlic is a perennial bulb forming plant that belongs to the genus *Allium* in the family Liliaceae is well known for having antibacterial effects.<sup>1,4</sup> For several centuries, garlic has been known to possess dietary and medicinal properties.<sup>5</sup> Garlic (*Allium sativum*) has come to be seen as an all rounded treatment for preventing wound infection, common cold, malaria, cough, lung tuberculosis, hypertension,

sexually transmitted diseases, mental illness, kidney diseases, liver diseases, asthma, and diabetes.<sup>6</sup> Though we have many antibiotics, we are becoming helpless due the emergence of bacterial resistance. The existing anti microbial agents have declined in effectivity due to resistance of organism to those agents.<sup>9,10</sup> This resistance is particularly evident in *Staphylococcus aureus*<sup>9,10</sup> and enteropathogenic bacteria, eg, *Shigella*, *Escherichia coli* and *Salmonella* species<sup>4</sup>.

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Because of the magnitude of the problem of drug resistance, some researchers have chosen to develop alternative strategies.<sup>11</sup> For several centuries, garlic has been known to possess dietary medicinal properties.<sup>12</sup> Several studies have proved that garlic has antimicrobial effects.<sup>13-16</sup> It inhibits the growth of both gram-negative and gram-positive bacteria, the same as molds and yeasts.<sup>17,12</sup> Increased consumption of *Allium* vegetables decreases the risk of gastric cancer possibly because of the effect of garlic on *Helicobacter pylori*, as this organism is associated with gastric cancer.<sup>18</sup> The antimicrobial activity of garlic has been attributed to the presence of thiosulfonates (eg, Allicin) whose removal completely renders garlic ineffective against microorganisms.<sup>19</sup> Allicin is obtained by crushing or cutting garlic cloves. The odorless amino acid, allin, present in the garlic cloves, is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to allicin and other thiosulfonates, which besides their antimicrobial effects, produce the characteristic odor of garlic.<sup>27</sup> Allicin acts by totally inhibiting RNA synthesis partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin.<sup>7</sup> Bacterial susceptibility to garlic may also depend on structural difference of bacterial strains. The cell wall of gram-negative bacteria contains 15-20% polysaccharide and 10-20% lipid, whereas gram-positive bacteria contain 35-60% polysaccharide and only 0-2% lipid.<sup>21</sup> The cell membrane of *Escherichia coli* contains 20% lipid, whereas *Staphylococcus aureus* contains 2% lipid.<sup>11</sup> The polysaccharide and lipid contents of the cell wall have an effect on the permeability of allicin and other garlic constituents. This may be responsible for the difference in susceptibility to garlic.<sup>18,11</sup> Garlic has long been used medicinally, most recently for its cardiovascular, antineoplastic and antibacterial properties. A wide range of microorganisms including bacteria, fungi,

protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Moreover garlic has been reported to reduce blood lipids and to have anti hypertensive properties. Most commonly, garlic is known for having a very positive effect on the immune response. It enhances the activity of phagocyte and lymphocyte cells which are responsible for locating and killing off foreign cells unwanted in the body. Taking garlic can not only increase immunity against the common cold, but it can also help increase recovery time. Considering all the matters, the present study was conducted to investigate antibacterial activity of *Allium sativum* (aqueous extract) against *Staphylococcus aureus*.

### Methods

The interventional study was performed in the department of pharmacology and therapeutics in collaboration with the department of Microbiology, Mymensingh Medical College, Mymensingh during the period July 2013 to January 2015.

**Collection of Garlic:** Garlic examined in this study was purchased from local market at Mymensingh, Bangladesh.

**Test organism:** Standard strain of *Staphylococcus aureus* ATCC 25923 was collected from Department of Microbiology, Mymensingh Medical College, Mymensingh.

**Preparation of Aqueous garlic extract:** For aqueous extract garlic cloves were peeled then cut into pieces then kept in shaded room for 7 days for drying and grounded in the grinder. 10 grams of the grounded garlic mixed with 200 ml of D/W by using mixer and leaved in room temperature for 24 hours. After that filtered by using gauze pad to remove the large particle then centrifuged at 3000 rpm for 10 minutes. Secondly by filter paper to obtain a clear solution. Then the

solution dried at and 40°C in hot water bath and was stored in the refrigerator until use. For preparation of parent solution by taking 1gm powder extract mixed with 10 ml D/W then filter by membrane filter ( pore size of the membrane was 0.45 micron) to eradicate the bacterial contamination. This solution was the source of preparing different concentrations. The extract was stored at 4°C in refrigerator.

**Preparation of Blank Antibiotic Disc:** With the help of punch machine Whatman No 1 filter paper was punched. The punched small filter paper was considered as blank discs. Then the discs were autoclaved.

#### Experiment:

Antibacterial sensitivity testing of Aqueous Garlic Extract (AGE) against *Staphylococcus aureus*

**Procedure:** Antibiotic sensitivity test was performed by Kirby-Bauer's Disc diffusion technique as following. A sterile cotton swab was dipped into bacterial suspension by matching to 0.5 McFarland tube for each isolates and streaked it in three directions on the surface of NA plates then left 5-10 mins

on room temperature. By using a sterile forceps the blank discs were placed on the surface of the plates. Then with the help of micropipette different concentrations of AGE were put over the blank discs and left for five minutes. Then the plates were incubated at 37°C for 24 hours then the zone of inhibition were measured in mm by using ruler.

#### Calculation of concentration of different AGE Disc Diffusion solutions:

Powdered garlic extract 1gm in 10 ml D/W. This solution was marked as **Parent solution** 10 ml D/W contain 1gm = 1000 mg aqueous garlic extract,

So, 1 ml Solution contain 100 mg AGE, This solution was marked as **Stock AGE DD (Disc Diffusion) Solution-I**

Then 1:10 dilution was done of stock AGE DD solution-I by adding 9 ml of DW

So, 10 ml solution contained 100 mg of AGE

So, 1 ml=1000 µl solution contained 10 mg=10×1000 µg of AGE=10000 µg

Thus, **10 µl solution contained 100 µg of AGE**; this solution was used in Disc Diffusion Method and different lower concentration solutions (25 µg and 50 µg per 10 µl) were made from this by adding DW.

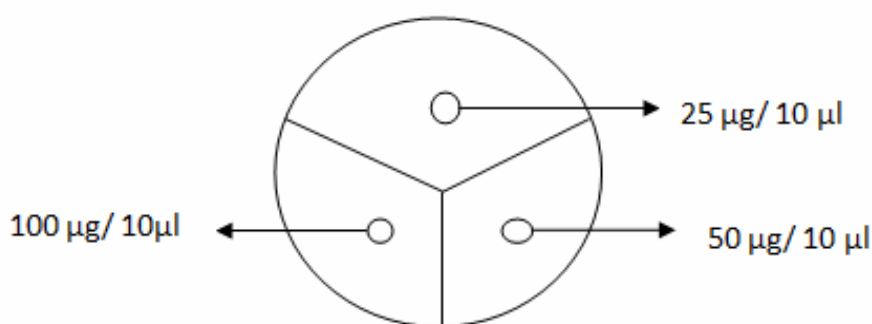


Figure 1: Sensitivity testing of AGE by disc diffusion method

## Results

Aqueous Extract in disc diffusion method sensitivity was seen against *Staphylococcus aureus* was 8 mm at 25 µg/10 µl, 14 mm at 50 µg/10 µl and 22 mm at 100 µg/10 µl concentrations (Table 1, Figure 2)

Table 1: Zone of Inhibition at different concentrations of AGE against *S aureus*

Concentration of AGE (µg/ 10 µl)	ZOI in mm
25	8
50	14
100	22

## Discussion

Aqueous Extract in disc diffusion method sensitivity was seen against *Staphylococcus aureus* was 8 mm at 25 µg/10 µl, 14 mm at 50 µg/10 µl and 22 mm at 100 µg/10 µl concentrations. It's declared that garlic, as an anti-bacterial agent, is effective against many more gram negative and gram positive bacteria like *Helicobacter pylori*, *Escherichia coli*, *Lactobacillus casei* and that this effect is sourced from allicin inside it.<sup>18,20</sup> It is also declared that components including sulphur in garlic and also bioflavonoids like quercetin and cyanidin in it have great value in preventing diseases and infections. It's revealed that active substances like allistatin I and allistatin II in garlic are powerful agents against staphylococcus and *Escherichia coli* bacteria.<sup>21-25</sup> It's predicted that antimicrobial effect of garlic has revealed in conclusion that thiosulphonates in garlic inhibit enzymes including thiol, which can take place in micro-organisms, as a result of the ability of quick reaction giving with thiol groups.<sup>22,26</sup> The antimicrobial activity of aqueous garlic extract against 15 Gram-negative and 2 Gram-positive bacteria with the help of agar well diffusion method was determined by

Srinivasan Durairaj.<sup>8</sup> In that study the zone of inhibition against *Escherichia coli*, *Pseudomonas aureginosa* and *Staphylococcus aureus* was 33 mm, 25 mm and 33 mm respectively at 100% concentration of AGE. In this study the zone of inhibition against *Staphylococcus aureus*, was 22 mm. In both studies it was found that AGE is sensitive against those test organisms. It's pointed out that garlic water has been used in typhoid and meningitis, its fume in whooping cough, garlic wicks in yeast infections and garlic soup in pneumonia. Garlic's this specialty possibly sourced from allicin components and being effective even against some organisms resistant to antibiotics necessitates its more use in standard medicinal applications<sup>22, 26</sup>. It is stated that garlic has very wide spectrum against gram positive and gram negative *Pasteurella*, *Coryne* and Micro-bacteria and this effect is seen in the bulb of garlic at the most. Furthermore, antibacterial spectrum of various garlic species shows difference. Antibiotic effect of garlic in watery extracts drops fast especially, at high temperatures. One of substances having strong antibiotic effect in it is allicin.

## Conclusion

From the study it is clearly observed that there is definite antibacterial effect of aqueous Garlic extract (AGE) against *Staphylococcus aureus*. Further studies are required to detect and isolate the active ingredients present in the Garlic extract responsible for antibacterial effect. Then their effects against the studied organisms should be studied in vivo separately and their toxicity profiles should also be taken into account. Only then the Garlic extracts fulfilled the criteria for its therapeutic use. Still then external application advised for burn and superficial skin infections and may be used in food poisoning, urinary tract infection and respiratory tract infection along with the conventional antibiotics which are used in those conditions.



Figure 2: Disc diffusion of AGE showing ZOI against *Staphylococcus aureus* at 100µg/ 10 µl of AGE solution with control

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