The Antibacterial effect of garlic's Extract on the Staph, Strep and E. coli Bacteria Species Isolated from Patients

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ABSTRACT

Over years, several studies have been made to overcome the emersion of resident species of bacteria. As far as the continually changing mechanism of the creation of the residence by bacteria, a permanent effort against the medicine residence is necessary. These days, detection and screening natural crops aiming to find novel effective compounds continues non-stop and in the recent years, some exquisite compounds with outstanding biologic activities have been achieved and the corresponding research area is expanding endlessly. The present study sought to examine the effect of garlic's aqueous extract on the resident strains of bacteria and comparing it with the conventional antibiotics. In the current study attempts have been made to investigate the effectiveness of garlic extract's antibacterial activity on Staphylococcus aureus Pseudomonas aeruginosa ESBL+, and E.Coli. To this end, based on the CLSI method, the third generation Cephalosporins were put 2.5 to 3 cm away from each other corresponding to the double disk method in a Muller Hinton environment then the Synergism effect was observed. The results revealed that it may be possible to introduce garlic's extract as a natural medicine with the least side effects and without the fear of getting bacteria resident to it as an alternative for the artificial antibiotics or to be used along with the common treatments to benefit the properties of that valuable plant.

INTRODUCTION

Garlic (Allium sativum) has long been known to have antibacterial, antifungal and antiviral properties but there are few data on its effects against bacterial species pathogens or their enzymes. In general, the minimal inhibitory and minimum bactericidal concentrations for the Gram-negative strains were lower than those for the Gram-positive strains tested (1,16). This project was conducted in order to study the effect of garlic on fighting Escherichia coli (E. coli) and the other bacteria. The testable question was “What is the effect of garlic on bacteria?” E. coli is a bacterium. Bacteria are one-celled organisms. Most types of E. coli do not cause disease. However, certain types of E. coli can be deadly, such as E. coli O157:H7. Bacterial infections cause many serious illnesses. Therefore, the information gained in this study could be used for future medicinal purposes in battling bacterial infections. Traditional healers have long used plants to prevent or cure infectious conditions. Western medicines trying to duplicate their successes(2,10) The antimicrobial activity of garlic has been attributed to the presence of thiosulfinate materials (e.g: allicin) in which their complete removal render garlic to be ineffective against microorganisms(3,14) The odorless amino acid allicin present in the garlic cloves is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to allicin and other thiosulfinates, which besides their antimicrobial effects produce which is a characteristic odor of garlic. Allicin acts by inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin; because of the magnitude of the problem of drug resistance,
some researchers have chosen to develop alternative strategies (17), which notes that garlic has broad spectrum activity and is known to act synergistically with antibiotic(4). Garlic contains at least 33 sulfur compounds, several enzymes, 17 aminocids and minerals such as selenium (5,13).It contains abiger concentration of sulfur compounds than any other Allium species. The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude and aged garlic extract (6,11). Studies have shown that, garlic also provides protection to the cardiovascular system by inhibiting platelet aggregation, protecting bloodvessels and lipoproteins from damaging effects of free radical oxidation and reducing serum cholesterol levels by inhibiting cholesterol synthesis(7,8)

Materials and Instruments:

Fresh garlic extract taken from Marynaj in Hamadan, 8 meters plate, Muller Hinton Agar and Moler Hinton Broth the 10 centimeters glass tube, the Wathman 42 filter paper, the 5cc and 10cc syringe, the acerate sampler, the blank disk, disks containing Oxacillin

The procedures of making the aqueous extract of garlic:

The aqueous extract of garlic was supplied based on the method followed by Mantis (manti). That is, after pealing the garlic, they were kept in the refrigerator for 24 hours and when having a fracture in each of them, they were left in the laboratory atmosphere, then using a mixer and a definite amount of sterile distilled water (one ml gram distilled water for per gram of garlic) a mixture of garlic was prepared. The mixture was passed through a sterile strainer and the 42 Wathman filter and the product was centrifuged in iceman centrifuge in 500 rpm orbit for 30 minutes. By which the redundant materials such as the cellulosic substances and the cell membranes were omitted the final product was a clear and yellow solution. Later it was sterilized by the 0.22 millipore filter and kept in the fridge for later uses.

At the first step, a new cultivation of Staphylococcus aureus resident to Methicilin (MRSA), Pseudomonas aeruginosa ESBL+, and E. coli was made. To this end, first the bacteria were cultivated from the stock environment to the Muller Hinton Broth as closed to flame, then they were incubated at 33-35 centigrade to reach an opaque of 0.5 Mc Farland (620 nm, 0.08-0.12). At the next step, from the achieved microbial suspension, by the help of a sterile swap and near flame were linear cultivated on the Muller Hinton Agar with standard PH=4.2-4.4 and 3-5mm diameter. On a plate consisting MRSA, one disk of Oxacillin (OX), one disk of pure garlic extract, and one blanket disk of sterile distilled as the control were put in 1.5 cm from the wall and 2 cm away from each other.

The anti-bacteria effect of garlic extract on bacteria:

The plates were incubated for 16-18 hours in 33-35 centigrade degrees and at the end the diameter of the no growth halo around the antibiotics and garlic were measured and compared by the transparent ruler. At the ending stage MIC of the extract was identified for all bacteria which were sensitive to garlic. To this end, dilutions of 1/4, 1/8, 1/16, 1/32, 1/64 v1/v2 were provided out of the achieved garlic extract. The process was that, first 6 sterile test tubes were chosen and one ml sterile distilled water was poured in each of them. Then one cc of the garlic extract, having passed from the syringe filter was added to the first tube and well mixed. At the next stage one cc of the mixture in the first tube was taken by the sterile sampler and was added to the second tube and then mixed with sterile distilled water and this was repeated to the sixth tube. Finally one cc of the mixture in the sixth tube was taken and thrown away. After achieving the desired dilutions, blanket disks were sank for the number bacteria separately. At the end from each dilution, one disk in 1.5 cm away from the wall and 2.5 cm from each other were separately put on the plate of each bacteria. Then the bacteria were incubated for 16-18 hours in 33-35 centigrade degrees and the minimum growth stopper density was determined for each bacteria according to the no growth halo of bacteria around the disks

Sample:

The Staphylococcus aureus resistant to methicillin, the Pseudomonas aeruginosa the producer of the broad-spectrum Betalactamase and the E.Coli bacteria the producer of the broad-spectrum Betalactamase. To this end, based on CLSI method, the third generation Cephalosporins were put 2.5 to 3 cm away each other corresponding to the double disk method in a Muller Hinton environment then the Synergism effect was observed. Resistance test to Oxacillin was performed to examine if Staphylococcus aureus is MRSA or not.

After each cultivation, the bacteria experience some changes in their genomes and there is a high possibility of mutation after being under some frequent cultures. That is, after several cultures, the bacteria's characteristics as their capacity for producing Betalactamase may convert. Therefore, to obtain more exact results in the experiments after the identification of the bacteria and making sure of their resistance, one culture called Stock from each of the bacteria were provided. In the current research, the effect of the aqueous extract of garlic on the MRSA, Pseudomonas aeruginosa ESBL+, and E. coli ESBL+ under the In Vitro circumstances has been studied and compared with common antibiotics.
Results:

According to the achieved MICs, the garlic extract has been effective on all the examined species. There was not a significant statistical difference between the effect of garlic’s extract and the effect of Co-amoxiclav, Sefti Zucsim, Cefotaxime, Vancomycin, and Oxacillin antibiotics. The only significant statistical difference there was between garlic and Ceftriaxone.

Table 1-5: Results of the effect of variant dilutions of garlic extract on MRSA and Sudomona AEROJINOAz in the disk placement method

<table>
<thead>
<tr>
<th>Garlic extract dilutions According v / v</th>
<th>The diameter of inhibition zone in mm around MRSA</th>
<th>The diameter of inhibition zone in mm around Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>1:4</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>1:8</td>
<td>14</td>
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<td>1:32</td>
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<td>1:64</td>
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The above results testify that garlic not only has a deterrence effect on the resident genera, but also has the same ability as the common antibiotics to restrain them.

The study made by Akhavan and his partners named the effect of garlic and cardamom’s extracts on the morphological and physiological properties of Staphylococcus aureus resident to Methicillin (MRSA) and Pseudomonas aeruginosa in Tehran, Recorded the effect of different dilutions of garlic extract on MRSA.

Pseudomonas aeruginosa as follows:

The results of the current study are in line with the research made by Akhavan since both prove the inhibitory property of garlic on MRSA and Pseudomonas aeruginosa and also in the case that the inhibitory property of garlic’ extract increases as the dilution raises. In the research done by Akhavan, garlic extract’s MIC for MRSA is the dilution 1/16 and for Pseudomonas aeruginosa is the dilution 1/8, but in the present research for the both species it is the dilution 1/2 which is in contrast with Akhavan’s results. On the other hand the non growth halo diameter at the dilution 1/2 in the Akhavan’s study for MRSA = 24 mm and for Pseudomonas aeruginosa =16. In
the present study, the non growth halo diameter at the dilution 1/2 for Pseudomonas aeruginosa =16 which is in line with Akhavan's study, but the non growth halo diameter for MRSA=12 that is in contrast with Akhavan's. The extract used in Akhavan's study is made of Hamedan's garlic by the Mantis method which is like this study. Also the method used to investigate the effect of the extract on the bacteria is the same Disk Diffusion method as used in the current study. That is, the difference between the results is probably due to the difference in the selection of MRSA and Pseudomonas aeruginosa species in the both studies and also the time and place of the studies. The strength of this study is the comparison made between the effect of the pure garlic extract with the effect of traditional antibiotics on the under study species and the examination on the ESBL+ species which have not been tested in the study made by Akhavan. In conclusion it may be deducted that garlic's extract can inhibit the resident species of bacteria as well as the common antibiotics. It may be possible to introduce garlic’s extract as a natural medicine with the least side effects and without the fear of getting bacteria resident to it as an alternative for the artificial antibiotics or to be used along with the common treatments to benefit the properties of that valuable plant.

Suggestions for Further Research:

The daily use of the edible garlic as an effective substance in remedy of the diseases caused by the resident species of bacteria or to prevent those illnesses in the consumers, needs some more comprehensive researches. Research on prescribing the combination of garlic’s extract with the common treatments of the resident species in comparison with the traditional treatments lonely in case of the rate of the effect, medicine's cost, and the duration of the treatment is suggested.

Limitations of the Study:

The most serious problem for the implementation of the current research was the risk of contamination by the resident species as there was the possibility of some dangerous diseases caused by such species.

REFERENCES
