The synergism effect of aqueous garlic extract and ciprofloxacin against some multi-resistant bacteria

Salah Salman Zain al-abdeen, Iman Tajer Abdullah and Sohaib Sabah Al-Salihii

Department of Biology, Science College, Kirkuk University, Kirkuk, Iraq

ABSTRACT

This study was aimed to evaluate the inhibitory effect of aqueous garlic extract (AGE) and synergism with two antibiotics Ciprofloxacin (CIP) and Ampicillin (Am) against 12 multi-resistant isolates, including two isolates of Staphylococcus aureus, three isolates of Pseudomonas aeruginosa and seven isolates of E. coli. Antibacterial activity of AGE was measured by using agar-well diffusion method at different concentration (200, 100, 50, 25 mg/ml). The results showed variable effect against the bacterial isolates, the highest inhibitory effect was seen against E. coli 7 and Staphylococcus aureus 2 as it reached to 25mm when 200 mg/ml concentration was used and the least effect was 8mm found against E. coli 5 by using the concentration 100 mg/ml. On the other hand the AGE did not show any inhibitory effect against Pseudomonas aeruginosa isolates. The synergism effect of the AGE and two antibiotics CIP & Am was studied by using double disc diffusion method. The results showed there is no any synergism between Am and the garlic extracts, while the CIP with garlic extract showed synergism effect in some bacterial isolates. E. coli (1, 3, 5, 6, 7) isolates showed synergism effect with inhibition zone ranged from (40-45), while the isolates 2 and 4 were resistant to the AGE, but it’s enhanced the inhibitory effect of the ciprofloxacin without any antagonism effect. The same thing was seen with Pseudomonas aeruginosa isolates. Finally the effect on the Staphylococcus aureus was additive which means the effect of the AGE and the CIP was equal to the summation of their effect together.

Key words: aqueous garlic extract, garlic & ciprofloxacin synergism, Bacterial resistance

INTRODUCTION

Increased using of alternative medicine in many developing countries which gave importance to use plants in the treatment[1,2]. Garlic is one of these plants which belonging to the family Liliaceae, it 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]. Historically, garlic (Allium sativum) had been found to possess many therapeutic properties including antimicrobial, antineoplastic, anti-cardio vascular, anti-hypertensive, anti-hyperlipidemia, anti-diabetic, immuno-stimulatory and hypoglycaemic activities[4-10]. Also, it has been used for centuries to fight infections for examples, Egyptians used it to treat diarrhea; the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections [11-15].

Louis Pasteur was the first describe the antibacterial effect of onion and garlic juices[16,17]. Garlic is a strong antibacterial agent against both Gram-positive and Gram-negative bacteria such as E.coli, Salmonella spp., Streptococcus spp., Staphylococcus aureus, Klebsiella spp., Proteus mirabilis, Shigelladysenteriae, Pseudomonas aeruginosa and Helicobacter pylori, [18-20] also it’s effective even against those strains that have become resistant to antibiotics[21]. Also, several studies indicated the effectiveness of garlic oils and juice against fungi, protozoa and viruses[22-28]. Sivam et al. was demonstrated the combination of garlic with antibiotics may lead to partial or total synergism[29].
Because of the increasing resistance of some bacteria to antibiotics. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better antibiotics against multiple resistant bacterial strains [30, 31].

Allicin, the active ingredient of *A. sativum* has been reported to have a range of potential targets. Firstly, inhibiting the acetylCoA forming system lead to inhibit DNA and protein synthesis, secondly inhibiting RNA synthesis as a primary target [32, 33]. The ability of bacteria to develop resistance to antibiotics in addition to the side effects of some of these antibiotics [34, 35], therefore the aims of this study were to investigate the effectiveness of aqueous garlic extract alone and synergism with two antibiotics. We used Ampicillin which its resemble garlic in its affectivity on cell wall synthesis in addition to influencing the synthesis of nucleic acids, which its resemble in effectiveness to the antibiotic Ciprofloxacin [36].

**MATERIALS AND METHODS**

**Isolation and diagnosis:**
Clinical samples (blood, urine and sputum) were collected in this study from children admitted to Kirkuk children's Hospital and the diagnosis of these isolates were confirmed in our laboratories depending on standard diagnostic procedures [37-39].

**Preparation of the bacterial suspension:**
Prepared by taking 3-4 colonies from each bacterial isolates to tube containing 5 ml of nutrient broth and incubated for 4-5 hours at 37 ° C and then added the physiological solution to it with shaking until they reached approximately to McFarland Standard tube No.0.5.

**Antibiotic sensitivity test:**
Antibiotic sensitivity test was performed by Kirby-Bauer’s disk diffusion technique [40] as following: A sterile cotton swab was dipped into bacterial suspension matching to 0.5 McFarland tube for each isolates and streaked it in three directions on the surface of Muller-Hinton agar plates then left 5-10 minutes on room temperature. By using a sterile forceps, the selected antibiotics (12 antibiotics 6 in each plate) was put on the surface of plate and left it for five minutes, incubated at 37 ° C for 24 hours then the zones of inhibition were measured in millimeter by using ruler.

**Preparation of aqueous garlic extract:**
Garlic cloves were peeled, cut into pieces and then kept in a shaded room for a period 7 days for drying and grounded in the grinder. Ten grams of the grounded garlic mixed with 200 ml of sterilized distilled water by using mixer and leaved it in the room temperature for 24hr. After that filtered firstly by using gauze pad to remove the large particles of the plants then centrifuged at 3000 rpm for 10 minutes, secondly by using filter papers to obtain a clear solution then dried in the oven at 40° C and stored in the refrigerator until used. For preparing stock solution by taking 2g of powder garlic extract and mixed with 10 ml D.W (concentration 200mg/ml) followed by filtration with membrane filters 0.45 micron to eradicate the bacterial contamination and this solution was used as a source for preparing different concentrations (100,50,25)mg/ml [13, 41].

**Effect of garlic aqueous extract:**
The effect of aqueous garlic extract was determined according to the agar –well diffusion method by working circular wells with diameter 6 mm in the surface of the Muller-Hinton agar plates by using cork borer with inoculated the surface of the media with bacterial suspension turbidity 0.5Macfarland standard, filled with 0.1 ml from each concentration of garlic extract then incubated in the 37 ° C for 24 hours. The zones of inhibition observed were measured in millimeters [33].

**Combination between extract and antibiotics:**
Sterile paper discs of 5mm diameter were impregnated with 10µl of last concentration of extract which gave significant inhibition effect against each bacterial isolates, then dried in a hot air oven at 40 ° C. Filter paper discs were placed on the surface of freshly inoculated Muller-Hinton agar plate beside it was put two antibiotics (ciprofloxacin & ampicillin). These plates incubated at 37° c for 24 hr. After the incubation the plates were examined for synergism effect by measuring the diameter of the zone in mm [42, 43].

**RESULTS**

**Sensitivity test:**
Twelve isolates were selected from three genus isolated from various pathological samples (Table 1).
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Table 1: Bacterial isolates from pathological samples

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Total number</th>
<th>Blood</th>
<th>Urine</th>
<th>Sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

The results showed that the most of these isolates possessed multiple resistant pattern to antibiotics, six (1,2,3,5,6,7) from the seven isolates of E. coli were resistant to seven or ten selected antibiotics except one isolate No.4 was identified as the fully susceptible to the all twelve antibiotics. (Table 2).

Table 2: The resistant pattern of isolated bacteriato antibiotics

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotics</th>
<th>SXT</th>
<th>DA</th>
<th>TE</th>
<th>AM</th>
<th>CIP</th>
<th>E</th>
<th>AMC</th>
<th>PRL</th>
<th>CL</th>
<th>TMP</th>
<th>PY</th>
<th>K</th>
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<td>R</td>
<td>R</td>
<td>27</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>10</td>
<td>20</td>
<td>R</td>
<td>R</td>
<td>16</td>
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<tr>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>35</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E.coli 3</td>
<td>R</td>
<td>R</td>
<td>20</td>
<td>R</td>
<td>26</td>
<td>15</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>E.coli 4</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>30</td>
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<td>10</td>
<td>30</td>
<td>10</td>
<td>30</td>
<td>20</td>
<td></td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>20</td>
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<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>15</td>
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<td>R</td>
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<td>R</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>20</td>
</tr>
<tr>
<td>E.coli 7</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>10</td>
<td>35</td>
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<td>10</td>
<td>27</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>25</td>
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<td>R</td>
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<tr>
<td>P. aeruginosa 2</td>
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<td>R</td>
<td>40</td>
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<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>P. aeruginosa 3</td>
<td>R</td>
<td>R</td>
<td>10</td>
<td>R</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>19</td>
</tr>
<tr>
<td>S.aureus 1</td>
<td>25</td>
<td>30</td>
<td>10</td>
<td>R</td>
<td>30</td>
<td>28</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>19</td>
</tr>
<tr>
<td>S.aureus 2</td>
<td>20</td>
<td>30</td>
<td>30</td>
<td>R</td>
<td>30</td>
<td>24</td>
<td>15</td>
<td>20</td>
<td>30</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>


TMP: Trimethoprim PY: Carbenicillin K: Kanamycin

The results showed the antibiotic CIP was effective against all isolates of E. coli and the zone of inhibition ranged between(20-40) mm and are represented the largest inhibition among antibiotics used and the least inhibition zones (10mm) was shown by the antibiotics {CL, PRL, AME, ER, AM, TE} (Table 2). Also the current study found that the two isolates(1 & 2)of Staphylococcus aureus were resistant to 3 and 4 antibiotics respectively, and the highest inhibition zone was recorded by using CIP and DA which reached 30 mm for two isolates, and TMP antibiotic used for isolates No. 1 and antibiotics TE and CL for isolatesNo. 2 and the least inhibition zone 10 mm was recorded by using TE antibiotics against the isolatesNo.1 (Table2)..

On the other hand, all strains of Pseudomonas aeruginosa was showed multiple resistance to antibiotics, it was found that all isolates were resistant to nine or ten antibiotics and the highest inhibition zone was recorded by using antibiotic CIP which reached 40 mm for isolate No.2 and least inhibition zone 10 mm was appeared by using TE antibiotic(Table 2).

The effect of aqueous garlic extract:
Antibacterial activity of the extract was tested against 12 isolates of bacteria which included two isolates of S. aureus, seven isolates of E. coli, three isolates of Pseudomonas aeruginosa with different multiple resistance pattern. There was variable effect of aqueous garlic extract concentration that used on the bacterial isolates, the most powerful effect was on E.coli7 and Staphylococcus aureus2 as it reached to 25mm when 200 mg/ml concentration used and the less effect of the same concentration to the E.coli3&5 which reached to 10mm, while by using 100 mg/ml, the strong effect was showed on the Staphylococcus aureus 2 it reached to 20mm and the less effect was on E.coli5 which reached to 8 mm in the same concentration and when 50 mg/ml concentration of the aqueous garlic extract used the inhibition zone was 10 mm only on the E.coli1&7 as shown in Figures 1, Fig.2(A)& Fig.3(A). This extract did not show any effect on the Pseudomonas aeruginosa isolates as observed in Fig. 1 & Fig. 4(A).
The synergism effects of the garlic extract with antibiotics:

By using the combination of the aqueous garlic extract with antibiotics the results showed there is no any synergism between Am and the garlic extracts, while the combination of the CIP with garlic extract showed positive effects as the action on the E. coli appeared to be same or more than the summation of the effect of each one alone as shown in Table 3 & Fig. 2(B). As for the Staphylococcus aureus isolates the effect was additive which means the effect of the AGE and the CIP was equal to the summation of their effect together (Table 3 & Fig. 3B). On the other hand isolates 1&3 of pseudomonas aeruginosa were resistant to the AGE, but it is strength the inhibitory effect of the ciprofloxacin (by increasing the inhibition zone of CIP from 25 to 40mm for pseudomonas aeruginosain1 and from 30 to 40mm for pseudomonas aeruginosain3 without any antagonism effect (Table 3 & Fig. 4B).

Table 3: The synergy effect of the AGE with two antibiotics (AM&CIP)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AGE</th>
<th>Am</th>
<th>AGE+Am</th>
<th>CIP</th>
<th>CIP+AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 1</td>
<td>10</td>
<td>R</td>
<td>-</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>E. coli 2</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>E. coli 3</td>
<td>10</td>
<td>R</td>
<td>-</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>E. coli 4</td>
<td>R</td>
<td>12</td>
<td>12</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>E. coli 5</td>
<td>8</td>
<td>R</td>
<td>-</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>E. coli 6</td>
<td>10</td>
<td>R</td>
<td>-</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>E. coli 7</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>P. aeruginosain1</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>P. aeruginosain2</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>P. aeruginosain3</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>S. aureus 1</td>
<td>15</td>
<td>15</td>
<td>-</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>S. aureus 2</td>
<td>15</td>
<td>15</td>
<td>-</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>

AGE=Aqueous Garlic Extract Am=Ampicillin CIP=Ciprofloxacin

Figure 1: Inhibition effect of the aqueous garlic extract against some bacterial isolates

Available online at www.scholarsresearchlibrary.com
Figure 2  A: The effect of aqueous garlic extract on *E.coli*, B: The effect of aqueous garlic extract and CIP on *E.coli*

Figure 3  A: The effect of aqueous garlic extract on *S.aureus*, B: The effect of aqueous garlic extract and CIP on *S.aureus*

Figure 4  A: The effect of aqueous garlic extract on *P.aeruginosa*, B: The effect of aqueous garlic extract and CIP on *P.aeruginosa*
DISCUSSION

This current study was aimed to detect the effectiveness of garlic aqueous extract toward number of bacteria which have multi-resistance characteristic against antibiotics and the antibacterial activity of garlic has been recognized centuries ago[44] and the inhibition zone that appeared was compared with the inhibition zone of Am & CIP. We selected ciprofloxacin antibiotic because all the bacterial isolates were sensitive for it, while Am selected as most of the bacterial isolates were resistant for it. The types of bacteria has been isolated (Table 1,2) are the causes of many diseases[45-49].

The present study found that most of bacterial isolates have acquired multiple antibiotic resistance (Table 2), this is a serious indication that need study this situation in details and determine the best antibiotic should be given to the patients infected by these bacteria, as increasing the numbers of bacteria resistance to the antibiotics is a real serious health problem throughout the worldwide[50] especially after increasing the problems that related to the penicillin and other antibiotics[51]. Although, the difference of the research places and the environment of the plants, the current study showed that aqueous garlic extract have an inhibition effect against the bacteria that had multiple resistant pattern (Fig. 1) and this result agree with the studies indicated the same results which showed that the garlic aqueous extract inhibit the growing of E.coli, Staphylococcus aureus and Pseudomonas aeruginosa[12, 52,53].

The combination of the aqueous garlic extract and ciprofloxacin which led to the synergism effect in some bacterial isolates (Table 3), this finding is similar to those reported by Sohn et al.[54] that appeared in five isolates of E.coli[1,3,5,6,7] this finding confirm that substances which affect bacterial nucleic acid shows synergism effect[55]. However, the isolates 2 & 4 resistant to AGE, but it is strength the affectivity of the ciprofloxacin this may be due to increasing the diffusion of this antibiotic to inside of the bacterial cells as the garlic may affect the cell wall of the microorganism.[36] The Same thing was seen with pseudomonas aeruginosa isolates, while the effect on the Staphylococcus aureus was additive which means the effect of AGE and CIP was equal to the summation of their effect together. Also, it did not show any synergism or antagonism effect with the CIP on the E.coli isolates 2, 4 and pseudomonas aeruginosa as occurred with E.coli used in the study of Eja et al.[53] that demonstrated the reduction of the effect of the CIP and garlic extract, while the comparison the effect of Staphylococcus aureus in the current study with that of Eja et al.[53] which showed less effect than the effect of two substances together, although our result was additive effect. Finally, the combination between the extract and Am not showed any synergism because of either the differences in the methods of extraction or the differences in nature of the bacterial isolates specially the composition of the cell wall which interfere with diffusion into the cells[29, 56].

CONCLUSION

1. Twelve isolates were selected from three genus isolated from various pathological samples (blood, urine and sputum). They are investigated by culture methods for the isolation.
2. Antibacterial sensitivity test was performed by Kirby-Bauer’s disk diffusion method and the majority of these isolates possessed multiple resistant pattern to antibiotics.
3. Antibacterial activity of AGE was measured by using agar-well diffusion method at different concentration (200, 100, 50, 25 mg/ml) and the highest inhibitory effect was seen against E.coli 7 and Staphylococcus aureus 2 as it reached to 25mm when 200 mg/ml concentration.
4. The synergism effect of the AGE and two antibiotics CIP & Am was studied by using double disc diffusion method. There is no any synergism between Am & garlic extracts while, CIP with garlic extract showed synergism effect with isolates (1, 3, 5, 6, 7) of E.coli with inhibition zone ranged from (40-45), while the isolates 2 and 4 of E.coli & pseudomonas aeruginosa were resistant to the AGE, but it's enhanced the inhibitory effect of the ciprofloxacin without any antagonism effect. Finally the effect on the Staphylococcus aureus was additive effect.

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