Study the Inhibitory Effect of Garlic Extract on *S. aureus* and *E. coli* of Multi-Resistance to Antibiotics.

دراسة التاثيرالمثبط لمستخلص الثوم على بكتريا المكورات العنقودية والأشريشيا القولونية المقاومة للمضادات الحياتية

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Abstract

This Study aimed to investigate the effect of garlic extract against multidrug resistance of *Staphylococcus aureus* and *E. coli* isolated from urine. Isolates were identified by using biochemical tests. The susceptibility of these microorganisms towards the most effective antibiotics were studied by using disc diffusion method. The multidrug resistance microorganisms were challenged with garlic extract using serial dilutions and the technology of ELISA spectrophotometer. The results of this study showed that ten isolates of *E. coli* and eight isolates of *Staphylococcus aureus* out of sixty isolates were resistant to all antibiotics. Garlic extract significantly inhibits the growth of these bacteria in a concentration independent manner. This study showed that garlic extract exerts a promising antibacterial effect against multidrug resistance of *Staphylococcus aureus* and *E. coli*.

Key word: Garlic, Antibacterial antibiotics, *Staphylococcus aureus* and *E. coli*.

الملخص

الهدف من هذه الدراسة هو التحري عن تأثير مستخلص الثومضد المكورات العنقودية الذهبية والاشريشيا القولونية المعزولة من عينات الادرار. شخصت هذه العزلات باستخدام اختبارات الكيمياء الحياتية المعروفة. اختبرت حساسية هذه المايكروبات نحو المضادات الميكروبية الاكثر فاعلية بطريقة الانتشار القرصي. الناتج من المايكروبات ذات المقاومة المتعددة للأدوية عرضت الى مستخلص الثوم باستخدام التخفيفات التسلسلية وتقنية الاليزا الطيفي. اوضحت نتائج هذه الدراسة عشرة من عزلات الاشريشيا القولونية وثمان من عزلات المكورات العنقودية الذهبية من أصل ستون عزلة كانت مقاومة لجميع المضادات الميكروبية. وان مستخلص الثوم يثبط وبشكل كبير نمو هذه البكتريا بطريقة مستقلة التركيز. هذه الدراسة أظهرت ان مستخلص الثوم يمارس وبشكل واعد تأثير مضاد لبكتريا المكورات العنقودية الذهبية والاشريشيا القولونية ذات المقاومة المتعددة للمضادات الحياتية.

الكلمات المفتاحية: الثوم, المضادات الميكروبية, المكورات العنقودية الذهبية، والاشريشيا القولونية

Introduction

Garlic (*Allium sativum*.L) is a plant that belongs to the genus *Allium* in the family Liliaceae. The antibacterial effects of garlic extended to inhibit of both Gram positive and Gram negative as well as molds and yeasts [1, 2, 3] Allicin, a thiosulfinate substance, and the amino acid allicin are the active substances that possessed antibacterial effect [4, 5]. The antibacterial effect of garlic mediated through inhibition of bacterial protein synthesis, RNA, partially DNA and even cell wall [6,7, 8]. Garlic inhibits the synthesis of cell wall in a mechanism that depended on the structure or the constituents of the cell wall. The antibacterial activity of garlic on the cell wall quantified to be 1 mg garlic is equipotent to 15 IU of penicillin [9]. Synergism or additive effect observed when garlic combined with ampicillin whereas the antibacterial effect of ciprofloxacin lost when combined with garlic [10]. Karuppiah [11] investigated the antibacterial activity of ethanol extract of garlic extract against multidrug resistant Gram positive and Gram negative and found effectiveness of garlic against these microorganisms except *Enterobacter* species and *Klebsiella* species [11]. The goal of this study is to avoid of using combination of garlic and synthetic antibacterial agents were the antagonism may occur. Therefore, this study aimed to investigate the antibacterial effect of garlic against bacteria isolated from human that possessed resistance to the traditionally synthetic antibacterial in *vitro* susceptibility testing.

Material and methods

This study was done in Department of Microbiology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq during May and June 2013. The study approved by the Institutional Scientific Committee. A total number of sixty urine samples collected from diabetic patients attended with urinary tract infection at Al-Yarmouk Teaching hospital.

Isolation, identification of the bacteria

The bacteria were isolated from diabetic patients with urinary tract infection by using the procedures described by Noda and Khadim [12], *Staphylococcus aureus* and *E. coli* were cultured on the Blood agar, EMB agar, Mac Conkey agar respectively and incubated for 24 hours at 37°C. The isolated bacteria were identified by biochemical tests[13]. A stock preparation of bacteria was done using glycerol and preserved at -20°C until use [14].

Antimicrobial susceptibility testing

Kirby-Bauer disc diffusion method was used for studying the sensitivity of the bacteria to various antimicrobials [15]. The antibiotics that used in this study (µg/disc) were: gentamicin (10), ciprofloxacin (5), nitrofurantoin (300), amoxicillin (30), imipenem (10), cefoxitin (30), clindamycin (30), piperacillin (100), tetracycline (30) and Co-trimemethaxazoloe (25). The inhibition zone was determined after incubation for 24 hour of bacteria cultured in nutrient agar at 37°C. The results interpreted according to the Clinical Laboratory and Standard Institute Guidelines [16].

Extraction of garlic

Fresh garlic obtained from local markets. 100 gram peeled garlic mixed by electrical machine , then the particles removed by passing the extract on the mesh-cotton tool, followed by filtration using Watt man filter paper number # 1.The extract sterilized using Millipore filter (0.45µm). The obtained aqueous kept at -20°C until used. A known volume of aqueous extract diluted 640 times before subjected to UV-Visible spectrophotometer scanning to identify the peak absorbance of the active ingredient. A maximum absorbance of 0.295 obtained at 335 nm wavelength.

Antimicrobial activity of garlic extract against resistant bacteria

A total number of eight isolates of each *Staphylococcus aureus* an d eight isolates of *E. coli* were challenged with the garlic extract. Microtiter plates were used in studying the antimicrobial activity of garlic. To each well, the broth, an inoculums of *Staphylococcus aureus* or *E. coli* and a known serial volume of garlic extract starting with 640X dilution (25µl). The Microtiter plates incubated overnight at 37°C. On the next day the absorbance of the cultured media recorded at630 nm using the technology of ELISA spectrophotometer. The antibacterial activity of garlic determined using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of cultured inoculum} - \text{Absorbance of the blank}}{\text{Absorbance of cultured inoculum in presence of garlic extract} - \text{Absorbance of blank}} \text{ x } 100$$

Results

Table (1) shows the susceptibility of *E. coli* to different groups of antibiotics. The highest percentage of susceptibility of *E. coli* observed with nitro furantoin which amounted 12(40%) and the lowest reported with tetracycline that amounted 2(6.7%). Ten out of thirty 10(33.3%) *E. coli* isolates were resistant to all tested antimicrobials.

Table (1): The antibiotic susceptibility of *E. coli* isolated from urine.

Antibiotics	Sensitive (mm)	Resistant	Susceptible	Total
Gentamicin(10µg)	≥ 15	21	9	30
Pipercillin (100)	≥ 21	23	7	30
Ciprofloxacin (5µg)	≥ 21	23	7	30
Nitrofuratoin (300µg)	≥ 15	18	12	30
Co-trimemethaxazoloe(25µg)	≥ 16	25	5	30
Tetracyclin (30 µg)	≥ 15	28	2	30

Table (2) shows the susceptibility of *Staphylococcus aureus* to different groups of antibiotics. The highest percentage of susceptibility of *Staphylococcus aureus* observed with ciprofloxacin which amounted 13(43.3%) and all the isolates are resistant to gentamicin. Eight out of thirty 8(26.7%) *Staphylococcus aureus* isolates were resistant to all tested antimicrobials.

Table (2): The antibiotics susceptibility of *Staphylococcus aureus* isolated from urine.

Antibiotics	Sensitive (mm)	Resistant	Susceptible	Total
Gentamicin(10µg)	≥ 15	30	0	30
Clindamycin (30)	≥ 21	24	6	30
Chloramphenicol (30µg)	≥ 18	28	2	30
Imipenem (10µg)	≥ 23	28	2	30
Ciprofloxacin (5µg)	≥ 21	17	13	30
Cefoxitin (30 µg)	≥ 26	23	7	30

Table (3) shows that garlic extract inhibits more than 90% of *E. coli* growth in four out of eight isolates that showed multi-drug resistant to the antibiotics. The growth inhibition of *E. coli* does not depend on the concentration of garlic.

Table (3): *E. coli* growth inhibition (%) induced by different concentrations of garlic extract.

Concentration (µg/ml)	25	12.5	6.25	3.125	1.562	0.781	0.39	0.195	0.0975
No. of isolates									
1	91.2	79.0	76.2	80.84	78.15	98.3	80.15	65.34	53.6
2	95.4	96.2	98.14	100	100	100	100	99.5	98.0
3	100	100	100	100	100	100	100	100	100
4	100	100	100	100	100	100	100	100	99.92
5	100	100	100	98.55	100	100	100	88.2	91.4
6	100	100	100	100	100	58.3	89.8	100	100
7	97.75	92.7	100	100	100	100	100	100	100
8	97.7	97.6	96	100	93.0	65.6	63.4	24.1	59.5

Table (4) shows that garlic extract inhibits the growth of multi-drug resistant *Staphylococcus aureus* in pattern not related to the dose dependent. The growth of five out of eight isolates that expressed multi-drug resistant completely inhibited with any concentration of garlic.

Table (4): *Staphylococcus aureus* growth inhibition (%) induced by different concentrations of Garlic extract.

Concentration (µg/ml)	25	12.5	6.25	3.125	1.562	0.781	0.39	0.195	0.0975
No. of isolate									
1	88.7	87.3	90.75	98.15	94.5	94.4	97.3	95.0	92.4
2	100	100	79.24	87.3	95.2	90.6	91.2	97.1	96.9
3	100	100	100	100	100	100	100	100	100
4	98.4	100	100	100	100	100	100	100	100
5	96.4	100	100	100	100	100	100	100	98.52
6	67.6	77.5	91.34	91.9	95.84	100	41.4	100	100
7	97.8	100	99	100	100	100	100	100	100
8	100	100	100	100	100	100	100	100	100

Discussion

This study showed that garlic extract is effective against multidrug resistant *Staphylococcus aureus* and *E. coli* isolates in a non-dependent concentration manner. The antibacterial effect of garlic extract against multidrug resistant gram positive or gram negative is equal. The results of this study are in agreement with other studies. In one case a study, reported the effectiveness of garlic as an adjunct therapy in management of infection due to multi-drug resistant *pseudomonas aureginosa* [17]. Another study reported that garlic extract inhibited both multidrug resistant and non-multidrug resistant *Streptococcus mutans* with a minimum inhibitory concentration ranging between 4 and 32 µg/mL [18]. The effect of garlic as antimicrobial extended to involve its effect against multidrug resistant *candida albicans* with a higher minimum inhibitory concentration than corresponding value against bacteria [19]. This study adds further information to the previous studies. First, the effectiveness of garlic

extract observed in both grams positive and negative, and second its effect can achieve even with small concentration. The isolates used in this study are resistant to multiple drugs of different mechanism of action. This leads to suggest that the effect of garlic extract against bacteria is not restricted to the one site like protein synthesis or inhibition of the cell wall synthesis. The antibacterial effect of garlic extract in this study can attribute to the active ingredient that observed at wavelength with a peak absorbance. Therefore, identification the nature and the chemical structure of this bioactive metabolite may disclose the mechanism of action of garlic. It concludes that garlic extract exerts promising antibacterial effect against multidrug resistant *E. coli* and *Staphylococcus*.

Conclusions

From this study and the earlier reports it is clear that, garlic appear to satisfy all of the criteria for antibacterial agents, being cheap and safe. Garlic can be utilized for the development of broad spectrum antibiotics as it has wide spectrum antibacterial activity and we concludes that garlic extract exerts promising antibacterial effect against multidrug resistant of *E. coli* and *Staphylococcus*.

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