

Comparative study of effect garlic and onion extract on the growth of some gram negative bacteria

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Abstract

During this study, a comparison of antimicrobial effects of garlic and onion extract on some gram negative bacteria were assayed in vitro. Primarily, each of these microorganisms including *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were isolated and diagnosed from patients suffering from different infection cases. This step was followed by preparation of aqueous extracts from onion and garlic. Furthermore, the activity of the two extracts on those microorganisms was tested by using different garlic extract concentrations, and application cup–plate diffusion and total count methods. It was noticed that increasing garlic aqueous extract concentration, would lead to a notable increase in inhibition zone diameter, compared with control. However the inhibition zone diameter was about 28 mm for *Salmonella typhi*, at concentration of 500mg/0.5ml of garlic extract was used; while the diameter was about 26–30–16 mm for the same concentration for each of *K. pneumonia*, *Escherichia coli* and *P. aeruginosa* respectively. Moreover a comparison was done between these results and that obtained when using onion aqueous extract. It was observed that the activity of garlic was more than onion which gave inhibitory zone diameter of about 25–16–17 mm to each of *S. typhi*, *K. pneumonia* and *E. coli* respectively , at concentration of 500mg/0.5ml; but the *P. aeruginosa* was unaffected. Other observation was show that there is adecrease in number of isolated microbial cells by increasing both extract concentrations, hence when treating the bacteria with garlic extract of 500mg/0.5ml concentration, the number of cells forming colonies were 62×10^2 , 71×10^2 , 12×10^3 and 13×10^3 cell/ml for each of *S. typhi*, *K. pneumonia*, *E. coli* and *P. aeruginosa* respectively. In the other hand, the number of colony forming unit for the same bacteria were 16×10^4 , 31×10^3 , 1×10^5 and 8×10^6 cell/ml when using onion extract of 500mg/0.5ml concentration.

Introdduction

Medical plants may be defined as any plant that can be put into culinary or medicinal use and also include those that are associated with orthodox drugs such as fox glove and opium poppy (Weinman *et al.*,1983) The onion is one of the oldest cultivated vegetables in history. Sulfur components in onion have also shown to be anti-inflammatory by both inhibiting formation of thromboxanes and by inhibiting the action of platelet – activating factor (PAF). Thiosulfinates components have anti-thrombotic benefits, including antioxidant activity, reduce serum cholesterol and enhance platelet activity in vitro. Flavonoids are a second class of health enhancing compound produced by onion and quercetin is one example. Flavonoids are chemical compounds active against microorganisms; they have been found in-vitro to be effective antimicrobial substances against a wide array of microorganisms. Equally, its antibacterial power is effective against preventing numerous intestinal problems that take place as a result of alteration in the intestinal flora. (Azu and Onyeagba) . The different concentration of superoxide anion will auto regulation of blood flow in cerebral circulation to it ability to increase resist of host body to different type of microorganism (Zagorace and xamana ,2005). Garlic has been known to possess

dietary and medicinal properties and several studies have proved that garlic has antimicrobial effects. It inhibits the growth of both gram-negative and gram-positive bacteria, which is the same for molds and yeasts (Ross *et al*,2001 ; Pai and Platt,1992). 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 (Hughes and Lawson ,1991). Allicin is obtained by crushing or cutting garlic cloves. The odorless amino acid allicin present in the garlic cloves is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to allicin and other thiosulfates, which besides their antimicrobial effects produce the 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 (Sivam ,2001), which notes that garlic has broad spectrum activity and is known to act synergistically with antibiotic. No resistance has been reported but more clinical studies need to be done to assess the use of antibiotic/garlic combination for bacteria that are difficult to eradicate. (Eja ,*et al*,2007) Garlic is active against some genus of bacteria such as the *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus sp.*, *Salmonella sp.* and *Proteus mirabilis*. It is also active against viruses such as herpes simplex, HIV and influenza.(Singh and Shukla ,1948) have repeatedly shown that garlic is active against strains of bacteria that are highly resistant to antibiotic. As far back as(Cavallito and Bailey ,1944) demonstrated that both garlic juice & allicin inhibited the growth of *Staphylococcus*, *Streptococcus*, *Bacillus*, *Brucella* and *Vibrio* species using fresh and vacuum – dried powdered garlic preparations which were also found to be effective against *Citrobacter*, *Klebsiella* and *Mycobacterium* species, in addition to *Staphylococcus*, *Streptococcus* and *Proteus* species (Sharma,1977 ; Elnisma,1983). Despite of many garlic trials, only a few have demonstrated efficiency in animal and human studies against the leading causes of dysentery. The drug resistant strains included *Shigella dysenteriae* type1, *Shigella flexeri*, enterotoxigenic *Escherichia coli* and *Vibrio cholerae*. In this study the aim was to carry out a comparative assessment of the antimicrobial effects of garlic and onion on some gram negative bacteria and show the direct effect of these medical plants on some pathogenic bacteria. This study also shows some uses of garlic and onion for the treatment of infections in order to solve the drug resistance problem.

Material &methods

1-Sample collection

The microorganisms used in this study were isolated from urine specimens which were collected from outpatients attending AL-Husseiny's children hospital in Karbala. The samples were collected for analysis using prescribed procedures in (Collee *et al.*,1996).

2-Microbiological analysis of the samples

The isolated bacteria were diagnosed biochemically depending of (Holt *et al.*,1994) and according to methods described by (Macfaddin ,2000).

1- Preparing aqueous extract of garlic & onion

a- Onion

The onions were washed with clean sterile distilled water and allowed to air dry for one hour. The outer covering of the onions were manually peeled off. The onion bulbs were separated and then were washed and extracted as prescribed procedures in (Azu and Onyeagba ,2007).

b- Garlic

Some amount of garlic cloves were taken and peeled off, 50gm of garlic was weighted and extracted as prescribed procedures in (Eja *et al.*,2007)

4-Testing for the antibacterial effects of raw aqueous extract of garlic and onion by cup-plate diffusion methods

The cup – plate diffusion method was carried out by using methods described by (Azu and Onyeagba ,2007).

5-Estimate of antibacterial activity of plant extract

10 folds dilution of PBS (Phosphat Buffer Saline) was prepared for each bacterial species with each plant concentration. The serial dilutions were then plated in medium plates and incubated at 37°C for 18 hours. The bacterial colonies were counted and the results were expressed as CFU (colony forming unit per ml of suspension) (Cotter and adly ,2001).

Results

The negative bacterial isolates were selected directly by culturing samples on Macconkey agar plates which was also used to differentiate lactose from non lactose fermenter; the isolates were diagnosed biochemically according to (Holt *et al.*,1994). The result of the antibacterial properties of the garlic extracts on the test organisms are shown in figure 1, which shows the zones of inhibition of bacterial growth by garlic extract in contrast to ciprofloxacin which is regarded as positive control and also shows increase in the zones of inhibition with increase in garlic concentration which were exhibited at 500mg/0.5ml for *E.coli* 30mm, *S.typhi* 28mm and *K.pneumonia* 26mm. When using the measurement of Colony Forming Unit (CFU) to illustrate the effect of garlic on the growth of bacteria, it clearly appears that the number of test bacteria were decreased with the increase in the concentration of garlic extract as shown in figure 2; that shows significantly a decrease in number of bacteria with an increase in garlic concentration to reach 62×10^2 , 71×10^2 cells at 500 mg/0.5ml while *E.coli* & *P.aureogenes* reach to 12×10^3 and 13×10^3 cells respectively.

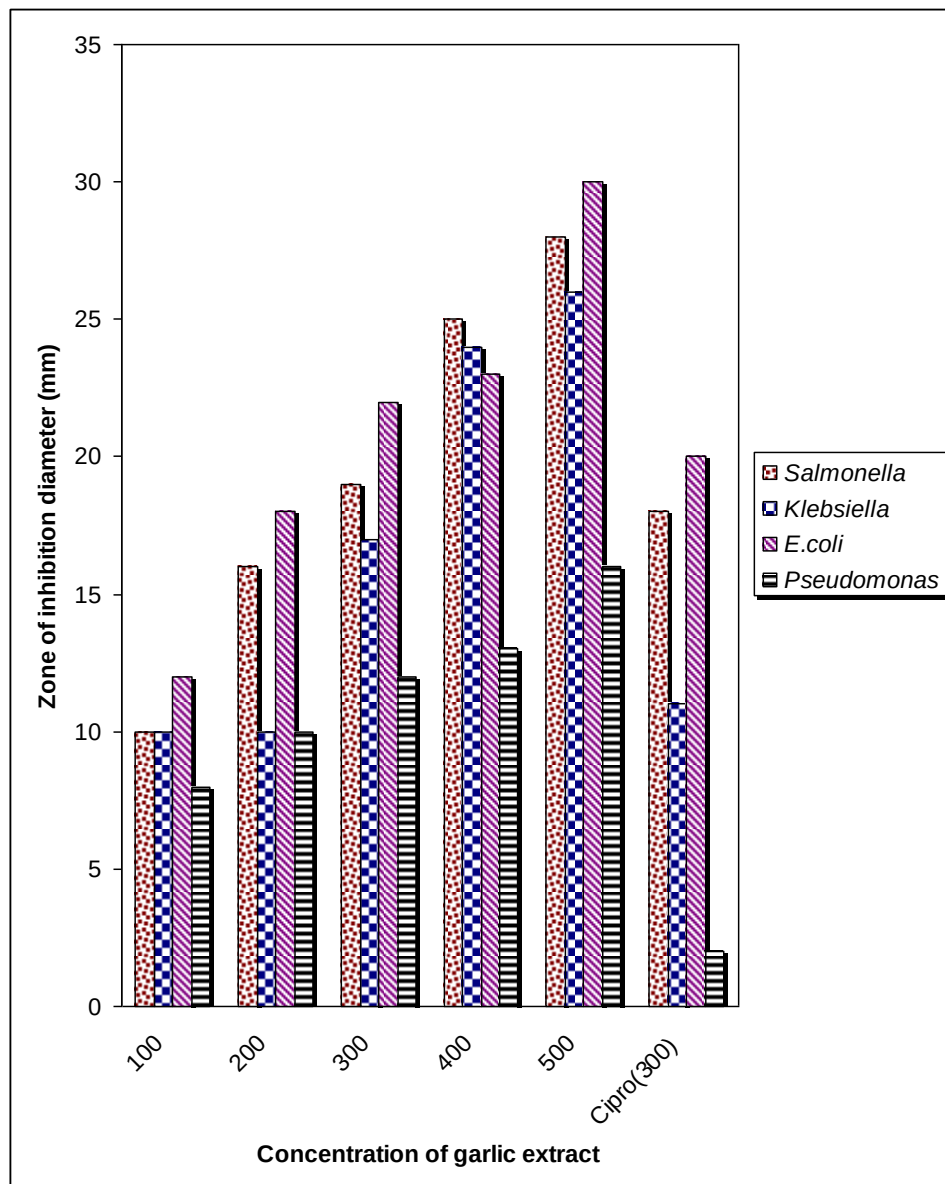


Figure 1: Antimicrobial sensitivity of M.O to garlic extract and Ciproflaxacin

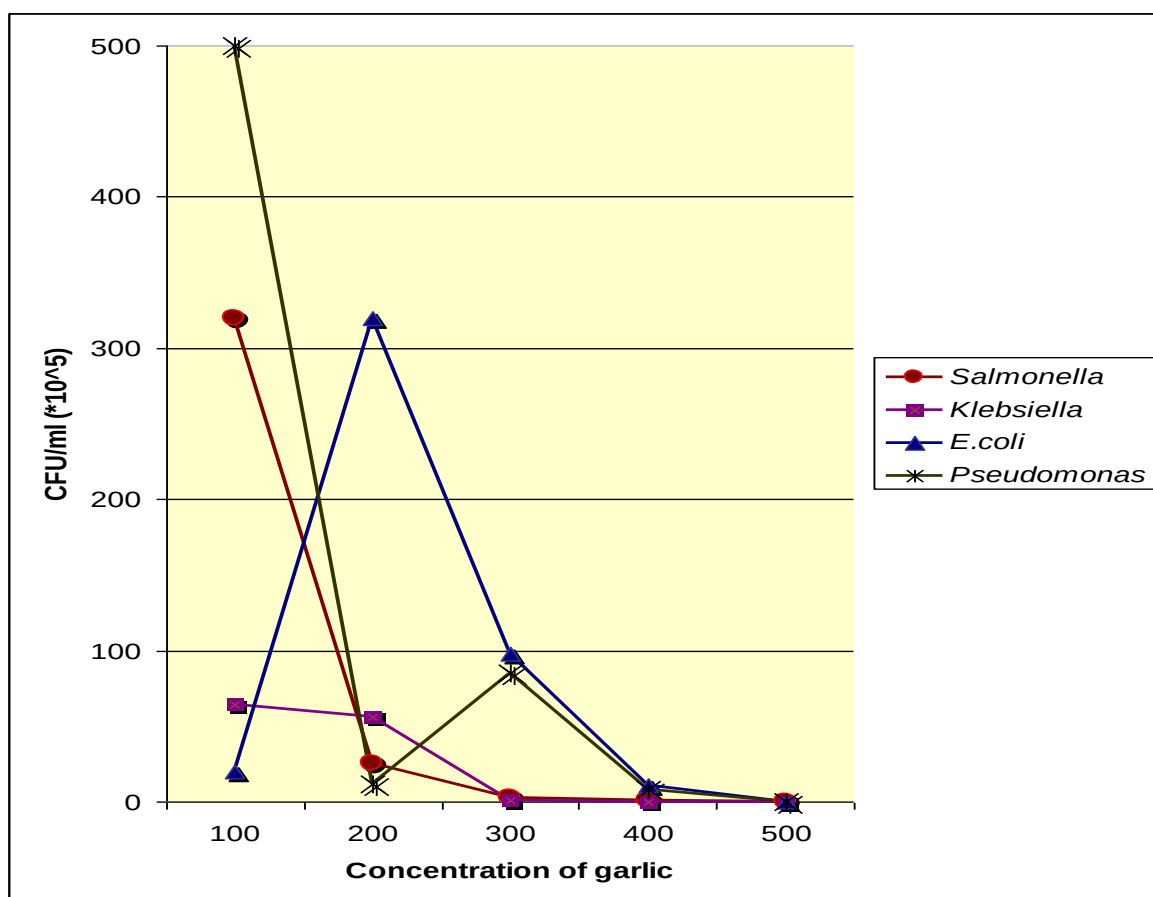


Figure 2: The number of test M.O treated with graded concentration of raw garlic extract

The study also observed zone of inhibition diameter of test bacteria to raw onion extract as shown in figure 3, that shows no effect on *P.aeruginosa* with different concentrations of onion but *S.typhi* and *E.coli* give obvious inhibition zone only at high concentrations that reached 17mm and 25mm respectively at 500mg /0.5ml, while *Klebsiella* showed increase sensitivity with increase in onion concentration which reached 16mm inhibition diameter at 500mg/0.5ml. This result was confirmed by measurement of CFU for each bacteria primed with graded concentration of onion extract as shown in figure 4.

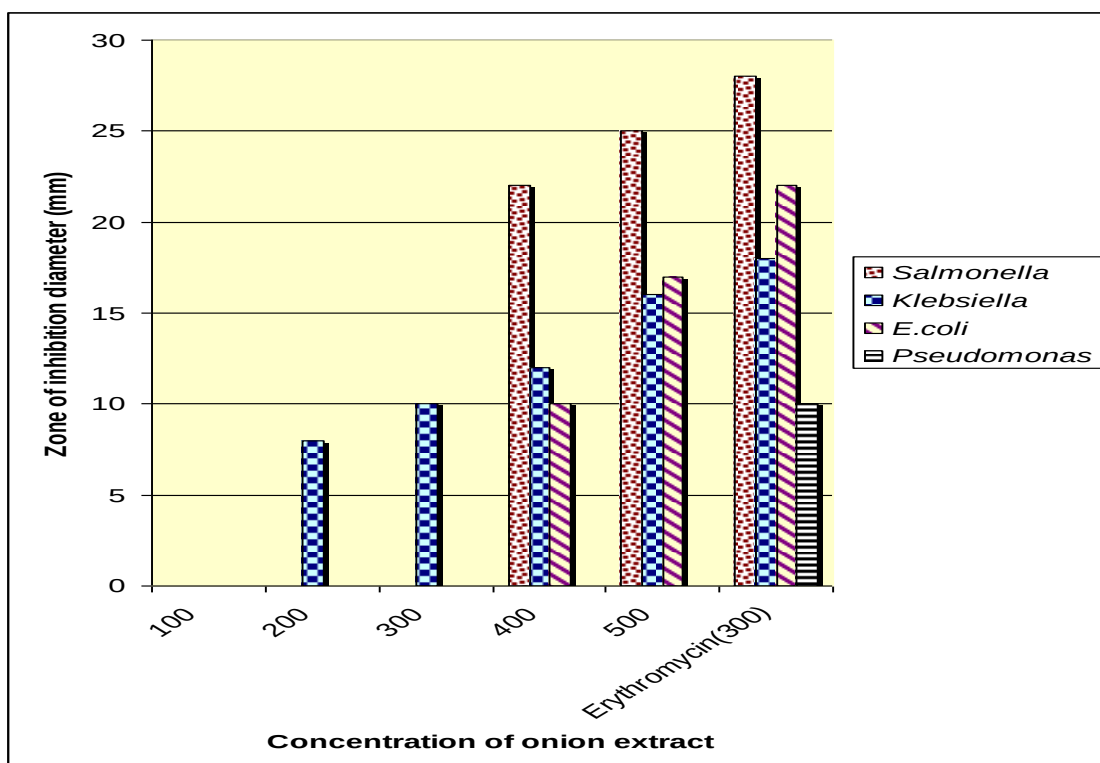


Figure 3: bacterial activity of extract onion & Erythromycin on test bacteria

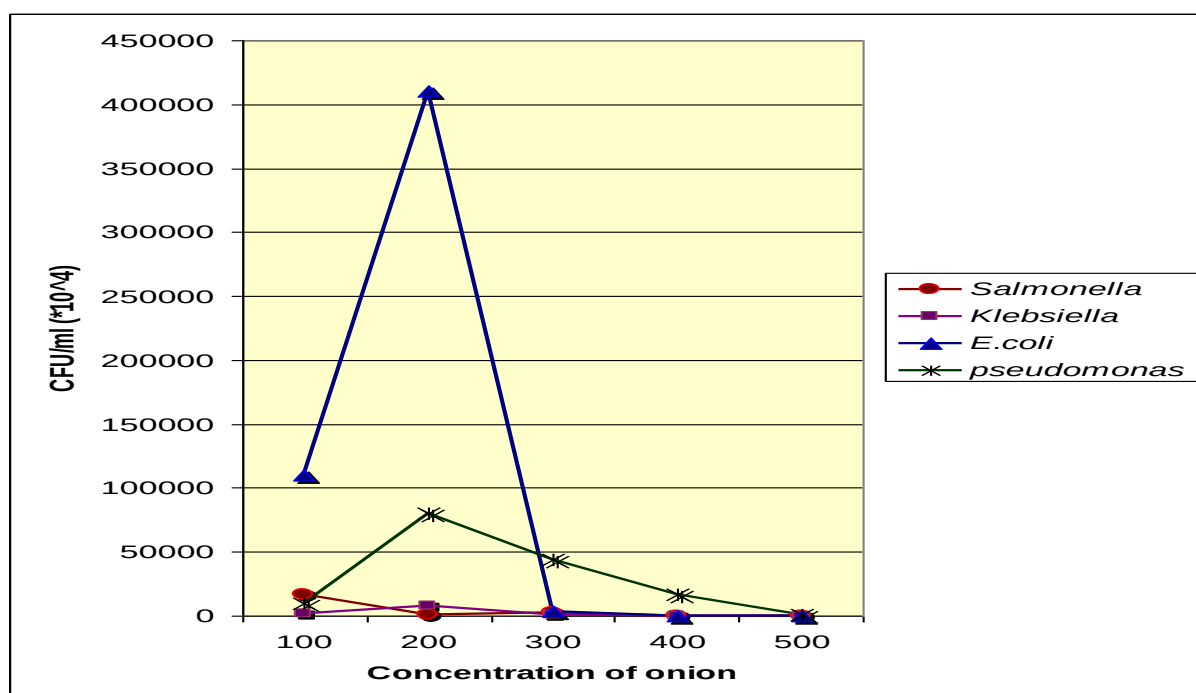


Figure 4: The number of test bacteria treated with graded concentration of raw onion extract

Discussion

It was clear from this study that the garlic showed to have antimicrobial activity on all the gram negative bacteria used, similar to CPX (Broad spectrum antibiotic) as seen in the results. These gram negative bacterial isolates were more susceptible to garlic, this was attributed to structural nature of cell wall of these

bacteria which contain 15–20% polysaccharide and 10–20% lipid and these materials affect the permeability of allicin and other garlic constituent, thus these bacteria observed susceptibility to garlic (Ross *et al*,2001).

The differences in the susceptibility of each test organism to the garlic extract may be directly related to the presence of secondary plant metabolites (thiosulfinates) and its concentration, in addition to the ability of microorganism to resist the antimicrobial agent that may occur because of multiple uses of antimicrobial agents in medicine. (Parker ,1978 ; Chen ,1985) *E.coli* and *Salmonella* species showed high sensitivity to garlic (Eja ,*et al*,2007) and this confirms the results obtained. The results also demonstrate that the effect of garlic extract on some gram negative bacteria depend on its concentration as shown in the figures. Moreover, garlic is more effective than onion and this may be attributed to the high molecular weight of the onion extract and the rate of diffusion is slowly reduced and also it takes longer time (Azu and Onyeagba ,2007). The sulfur compounds of onion have shown to be anti – inflammatory by inhibiting the action of platelet–activating factor (PAF) more than antibacterial effect (Parker ,1978). The bacteria used in this study were more sensitive to antibiotic than to onion extract, which could be arisen as a result of differences in the mode of action of the antibiotic and onion which have little antibactererial effect. (Chen ,1985) The highlight of this study was that garlic extract, which is more an effective antimicrobial agent than onion extract and antibiotics currently in use; therefore the use of garlic for the treatment of infections may be a solution for drug resistance problems. (Eja ,*et al*,2007)

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الخلاصة

أجرى خلال هذه الدراسة بيان مقارنة التأثير المضاد لنباتي الثوم والبصل على بعض البكتيريا السالبة لصبغة غرام خارج الجسم الحي. إذ تم عزل و تشخيص بكتريا *E.coli*, *S.typhi*, *K.pneumonia*, *P.aeruginosa* من مرضى يعانون من اصابات مرضية مختلفة. بعدها حضر المستخلص المائي لنباتي الثوم والبصل و بدأ بإختبار فعالية المستخلصين على تلك الجراثيم من خلال معاملتها بتراكيز مختلفة من تلك المستخلصات و بإتباع طرق مختلفة كطريقة الحفر و طريقة العد من خلال حساب عدد الخلايا المكونة للمستعمرات، حيث لوحظ وجود زيادة ملحوظة في قطر منطقة التثبيط مع زيادة تركيز المستخلص المائي للثوم مقارنة بالسيطرة حيث وصل إلى 28 ملليمتر عند التركيز 500 ملغم/0.5 مليلتر لجرثومة *S. typhi* في حين وصل إلى 16 – 30 – 26 ملليمتر عند نفس التركيز لكل من جرثومة *P. aeruginosa*, *E.coli*, *K. pneumonia* على التعاقب، و عند مقارنة هذه النتائج مع أقطار منطقة التثبيط الظاهرة من إستعمال مستخلص البصل لوحظ أن التأثير التثبيطي للثوم كان أكثر من البصل الذي أعطى أقطار تثبيطية وصلت إلى 16 - 17 ملليمتر لكل من جرثومة *E.coli*, *S. Typhi* و *K. pneumonia* على التعاقب عند التركيز 500ملغم/0.5 مليلتر، في حين لم تتأثر جرثومة *P. aeruginosa* عند هذا التركيز. الشيء الذي ظهر واضحا أيضاً هو الهبوط الملحوظ في عدد الخلايا الجرثومية المعزولة مع زيادة تركيز المستخلصين حيث وصل عدد الخلايا المكونة للمستعمرات 10×62^2 ، 10×71^2 ، 10×12^3 و 10×13^3 خلية/مليلتر لكل من جرثومة *P. aeruginosa* و *E.coli*, *K. pneumoni*, *S. typhi* على التوالي عند معاملتها بمستخلص الثوم و

بتركيز 500 ملغم/0.5 مليلتر ، في حين كان عدد الخلايا المكونة للمستعمرات للبكتريا نفسها 10^3 ، 10^4 ، 10^5 ، 10^6 و 10^8 خلية/مليلتر على التوالي عند معاملتها بالتركيز 500 ملغم/ 0.5 مليلتر من المستخلص المائي للبصل.