

THE USES OF GARLIC AS ANTIBACTERIAL IN VITRO

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ABSTRACT:The aim of this study was to determine the inhibitory effect of different concentrations of garlic extract in vitro. Thirty clinical isolates of *Staphylococcus aureus* were obtained from patients suffering from different skin infections in Ramadi General Hospital during the period between October 2007 to February 2008, as well as the control strain of *S. aureus* (ATCC 25923). Antimicrobial effects of garlic were detected by three different methods, disc diffusion method, well diffusion method, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Out of thirty clinical isolates of *Staphylococcus aureus*, 20 (66.6%) and control strain were identified as highly susceptible with 100mg/ml of garlic juice, 8 isolates (26.6%) showed intermediate susceptibility and no detectable activity with the lower concentrations of garlic juice as well as 2 isolates (6.6%) were resistant (no zone of inhibition) was observed with concentrated garlic juice. 3.2 mg/ml of garlic juice was the minimal inhibitory concentration and minimal bactericidal concentration for all tested isolates.

It is concluded that the higher concentration of garlic juice which had a strong inhibitory activity on *Staphylococcus aureus*. Further in vivo studies are necessary.

KEY WORDS :antibacterial ,garlic ,*S.aureus*

Introduction

The use of plants in medicine was gone as far back as thousands of years and

still continues today(1,2). In a review of its medicinal properties(3) reported that garlic possesses a range of antibacterial, antiviral, and antifungal activities, it decreases serum cholesterol and triglyceride concentration, lowers raised blood pressure levels, and is associated with reduced incidence of some form of tumours. The chemistry of

garlic has been described by (4) the species contain a number of sulphur-containing compounds, one of which is the odourless molecule. When the plant is cut or damaged, alliin comes into contact with the enzyme alliinase, which converts alliin into allicin (diallyl thiosulphinate or 2-propenyl 2-propenethiol sulphinate)(5,6). The latter is responsible for the typical smell of garlic, as well as many of its medicinal properties. In microorganisms, allicin interferes with lipid synthesis and RNA production. The target enzyme with which allicin interact

has been identified as acetyl-CoA synthetase, and (7) allicin has been used to synthesize ajoene. A compound with many of the properties of allicin; additionally, it has particular anti-fungal and anti-thrombotic effects (3). This study was carried out to determine the antibacterial effect of garlic extract in vitro.

Materials and Methods:-

Isolation of Bacteria :-

Thirty clinical isolates of *Staphylococcus aureus* and one control strain (The central of health laboratory, ATCC 25923) were tested. All 30 clinical isolates were obtained from patients suffering from different skin infections in Ramadi General Hospital. All study isolates were well bacteriologically identified and confirmed by biochemical tests(8). Bacteria were stored in brain heart infusion broth (BHI) medium containing 20% glycerol. Before each experiment, one aliquot was thawed quickly at 37°C and subcultured on Blood agar (BA, Difco) plates at 37°C for 24 hours.

Antibacterial activity assays were carried out using Brain Heart infusion Agar (BHIA ,Himedia) and Muller-Hinton broth(MHB ,Himedia).

Bacterial standardization:-

Bacterial inoculation was standardized according to McFarland suspension(9).

Tub no. 5 contain 1×10^5 cfu /ml one ml from this Tube was used as inoculum

- Crude extracts of Garlic preparation:-

100g of Iraqi fresh garlic was purchased from popular market ,were peeled and the cloves were pressed through a garlic press into an Atomix blender (ELARABY BLENDER MX-5100) which contain 100 ml of sterile water and allowed to stand

for 72 hrs . The crude extract was obtained by filtration , this extract was sterilized by passing through amillipore filter with a pore diameter of 0.45 mm (9).

The results are:-Four different concentrations .

(100,75,50,25)mg/ml from crude extract was used in the antimicrobial test (desic and well diffusion methods) .In MIC and MBC methods was used crude extract concentration 100% and prepared numbers of subsequently dilution with sterile water (90%-80%-70%-60%-50%-25%-12.5%-6.3%-3.2%-1.6%) .

Stored at 4°C to be used.

-Antimicrobials activity test :-

1- Disc diffusion method:-

One ml of bacterial suspension from tube no. 5 containing 1×10^5 cfu /ml was diffused evenly on BHI agar plate and kept in incubator at 37°C for 2 hours to be dry .Then sterile filter paper discs were impregnated with 0.1ml of sterile garlic extract concentrations . Those were allowed to dry for 10min in an open sterile

Petridish.Using aseptic procedures ,control assay discs impregnated with sterile water .Assay discs were placed on the surface of the inoculated Brain heart infusion agar.

plates were incubated at 37°C for overnight to 24 hours(10).

2-Gel diffusion method :-

one ml of bacterial suspension from tube no. 5 containing 1×10^5 cfu /ml was diffused on BHI agar plate.Circular wells (6mm×3mm)were cut in the agar culture media and full with 100µL of garlic extracts,control well was filled

with sterile water,these were inocubated at 37° C for over night to 24 hours(11).

3-Minimum inhibitory and bactericidal concentrations of garlic juice:-

Standered methods based on those of (12). were used to detrimine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) .Different garlic Concentrations (1-100 mg/ml) were prepared in 5 ml of Muller-Hinton broth and transferred to sterile capped .At least 4-5 morphologically similar colonies were inoculated into Muller-Hinton broth and incubated at 37°C until the viable turbidity was equal to the 0.5 McFarland,(about 108cfu/ml).After that, the suspension was diluted 1:100 and certain volumes transferred to the tubes containing garlic juice dilutions ,to reach a final cell concentration of (about 105 Negative cfu/ml).Negativ controls were represented by two tubes ,one of them contained broth only and the other contained broth plus microorganism.Then the tubes were incubated overnight at 37°C.The result of minimal inhibitory concentration (MIC) was interpreted as the lowest concentration of garlic which inhibits visible bacterial growth after overnight incubation .Subcultures of these were used to determine MBCs(12).

Results

The control strain produced a (30mm) diameter zone of inhibition to garlic . Out of 30 clinical isolates ,twenty isolates (66.6%) were identified as highly susceptible zone size (25mm-30mm) , eight isolates (26.6%) showed intermediate susceptibility and two isolates (6.6%)

were resistente no zone of inhibition .Results of antimicrobial activity assays indicated that garlic juice had inhibitory activity on S.aureus which is an important pathogen has been identified as the most sensitive against garlic espially at concentration (100and 75) mg/ml. Results of antimicrobial activity assays are represented in Table 1 ,Figure1,2 ,shows the antibacterial effects of various concentrations of garlic extract with two differet methods .Minimum inhibitory and bactericidal concentrations results showed the control isolate tested gave an MIC and MBC of 3.2mg/ml.The MICs and MBCs for all the

clinical isolates tested were either 1.6mg/ml or 3.2mg/ml.

Discussion:-

In this study, the purpose was to examine the inhibitory effects of different concentrations of garlic extract with four different methods in vitro, thirty clinical isolates of *S.aureus* were tested for this purpose. Examining findings, showed that the control strain and twenty isolates of *S.aureus* (66.6%) were observed highly susceptible against garlic juice, eight isolates (26.6%) showed intermediate susceptible and two isolates were resistance, the sensitivity of *S. aureus* to garlic extract agrees with earlier observations (13). Some investigators noted that sensitivity of microorganisms to chemotherapeutics differs according to type of strain (14). In this study, the various concentrations of garlic extracts displayed a variable degree of antimicrobial activity on *S.aureus*

results findings, showed the widest inhibition zone was formed around *S.aureus* (25mm-30mm) at concentration 100mg/ml with two methods (Disc and Well diffusion methods), the least inhibitory effect was observed at concentration 75mg/ml, results in agreement with that of (15,16). And lower dilution concentrations had no antibacterial effect, since that allicin can react with water to form diallyl disulphide (4,17), which does not the same level of antibacterial activity as dose. Several components, such as allicin and other thiosulfinates that are the source of the characteristic odor of garlic. Thiosulfinates and other secondary metabolites of garlic, including 7-glutamylpeptides, scordinins, steroids, terpenoids, flavonoids and other phenols, may be responsible for the range of therapeutic effects reported for garlic, have been isolated and characterized (15,18,19).

In the present study, 88% of isolates had MICs for garlic of 1.6mg/ml, and all isolates were inhibited and killed by garlic at 3.2mg/ml. This compares well with (15), reported tests on a single strain of *S.aureus* and noted that pure allicin had an MIC of 2.7mg/ml.

The inhibitory effect of garlic is thought to be due to allicin (4), the S(=O)S thiosulphinatate group in allicin is thought to react with a variety of SH-containing

enzymes within the bacterial cell, and allicin has been reported to have a range of potential target. It is reported to inhibit the acetyl CoA forming system, to inhibit DNA and protein synthesis, and to target RNA polymerase (20,21), and these are responsible for the agents antibacterial effect. More general proposals about the broad-spectrum activity of allicin were provided by (22). This group compared the importance of its antioxidant properties with its thiol disulphide exchange activity and suggested that activity is related to allicin's rapid reaction with thiol-containing protein. (23). Further in vivo studies are necessary

More importantly there is need for detailed scientific study of traditional medical practice to ensure that valuable therapeutic knowledge of some plants are preserved and also to provide scientific evidence for their efficiencies.

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References

- 1-Arora, D., J. Kaur. (1999) .Antimicrobial activity of spices ,*Int J.Antimicrob.Agents* 12:257-262
- 2-Cavallito, C., J. Bailey. (1994).Allicin,the antibacterial principle of *Allium sativum*.Isolation, physical properties and antibacterial action.*J.Am.Soc.*66:1944-1952.
- 3-McElnay,J.C. and Li Wan Po,A. (1991).Dietary substances (8) garlic.*Pharmaceutical Journal*,246,324-326.
- 4-Block, E.(1992).The organosulphur chemistry of the genus *Allium*:implications for the organic chemistry of sulphur.*Angew chem. Int Ed Engl*,31:1135-78
- 5-Chen, G .W., Chung, J.G.,Ho, H.C.,Lin, J.G.,(1999).Effect of the garlic compounds dially sulphide on arylamine N-acetyltransferase activity in *Klebsiella pneumonia*.*J.Appl.Toxicol.No.2*:75-81
- 6-Chung, J.G., Chen, G.W.,Wu, L.T.,(1998).Effect of garlic compounds diallyl sulphide and diallyl disulphide on arylamine N-acetyltransferase

- activity in strains of *Helicobacter pylori*. *Am. J. Chin. Med.* 26, No.3-4:353-64.
- 7-Focke, M., Feld, A., Lichrenthaler, K. (1990). Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-coA synthetase. *FEBS Lett* ;261:106-8
- 8-Baron, E.J., Peterson, L.R., Finegold, S.M. (1994). Method for testing antimicrobial effectiveness. In: *Diagnostic Microbiology, Bail and Scotts*. Toronto: C.V. Mobsy company., 9th ed: 168-193.
- 9-Onyeagba, R.A., Ugbogu, O. C., Okeke, C.U. and Iroakasi, O. (2004). Studies on the antimicrobial effect of garlic (*Allium sativum* linn), ginger (*Zingiber officinale* roscoe) and lime (*Citrus aurantifolia* linn). No.10, 552-554.
- 10-Vandepitte, J., El-Nageh, M., Tikhomiros, E., Stelling, J.M. (1996). Guidelines for antimicrobial resistance surveillance. World Health Organization. Alexandria, Egypt.
- 11-Andrews, J.M. (2001). BSAC standardised disc susceptibility testing method. *Antimicrob Chemother* ;48(suppl 1):5-16
- 12-Lorian, V. (1991). *Antibiotics in laboratory medicine* (3rd ed.), Williams and Wilkins Baltimore USA.
- 13-Ankri, S., Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and infection*. 1, No.2:125-9
- 14-Rees, L.P., Minney, S.F., Plummer, N.T., Slater, J.H., and Skyrme, D.A. (1993). A quantitative assessment of the antimicrobial activity of garlic. *World Journal of Microbiology and Biotechnology*, 9, 303-307.
- 15-Hughes, B.G., Lawson, L.D. (1991). Antimicrobial effect of *Allium sativum* L (garlic), *Allium ampeloprasum* (elephant garlic) and *Allium cepa* L (onion), garlic compound and commercial garlic supplement products. *Phytotherapy Research* ;5:154-8
- 16-Mangamma, P. and Sreeramulu, A. (1991). Garlic extract inhibitory to growth of *Xanthomonas campestris* pv. *Vesicatoria*. *Indian phytopathology*, 44, 372-374.
- 17-Lawson, L.D. and Wang, Z.Y.J. (1995). Changes in the organosulphur compounds released from garlic during aging in water, dilute ethanol or dilute acetic acid. *Toxicol*;14:214
- 18-Gara, J. (1994). B-Lactamases: Current situation and clinical importance. *Intensive Care Med.*, 20:3:S5-S9.
- 19-Gould, I.M. (1994). Risk factors for acquisition of multidrug-resistant gram-negative bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.*, pp:S30-S38.
- 20-Feldberg, R.S., Chang, S.C., Kotic, A.N. (1988). In vitro mechanism of inhibition of bacterial cell growth by allicin. *Antimicrob Agents Chemother* ;32:1763-8
- 21-Ozolin, O.N., Uteshev, T.A., Kim, I.A., Deev, A.A., and Kamzolova, S.G. (1990). Specific modification of the alpha-subunit of *E. coli* RNA polymerase by monomeric mercuric acetate. *Mol Bio (Mosk)*, 24:1057-66
- 22-Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D. and Weiner, L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochem Biophys Acta* ;No.2:233-44.
- 23-Ekweny, U.N. and Elegalam, N.N. (2005). Antibacterial activity of Ginger (*Zingiber officinale* Roscoe) and Garlic (*Allium sativum* L.) extract on *Escherichia coli* and *Salmonella typhi*. *Int. J. Mol. Med. Adv. Sci.*, pp:411-416

Table 1:Antibacterial effect of garlic extract on *Staphylococcus aureus*

Methods	Garlic extract concentration s	Inhibition zone diameters (mm)
2-Well diffusion method	1-100mg/ml	(25mm-30mm)
	2-75mg/ml	(12mm-20mm)
	3-50mg/ml	no detectable activity
	4-25mg/ml	no detectable activity
1-Disc diffusion method	1-100mg/ml	(25mm- 30mm)
	2-75mg/ml	(12mm-20mm)
	3-50mg/ml	no detectable activity
	4-25mg/ml	no detectable activity



Figure1 :Disc diffusion method



Figure 2:Well diffusion method

استخدام الثوم كمضاد حيوي في المختبر

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الخلاصة

اجريت الدراسة باستعمال 30 عزلة من بكتريا العنقوديات الذهبية *Staphylococcus aureus* جمعت من مرضى يعانون من الالتهابات الجلدية المختلفة في مستشفى الرمادي العام للفترة من تشرين الاول 2007 ولغاية شباط 2008, واستخدمت عزلة السيطرة (*S. aureus* (ATCC25923) لغرض المقارنة. استخدمت ثلاث طرق لدراسة الفعالية التثبيطية لمستخلص الثوم وهي (طريقة الانتشار حول القرص, طريقة الانتشار حول الحفر وطريقة قياس التركيز المثبط والقاتل الادنى).

أظهرت نتائج الدراسة بأن 20(66.6%) عزلة من بكتريا العنقوديات الذهبية بالاضافة الى عزلة السيطرة حساسية عالية عند التركيز (100)ملغم/مل لمستخلص الثوم , وظهرت 8(26.6%) عزلات متوسطة الحساسية عند التركيز (75)ملغم/مل لمستخلص الثوم, في حين لم تظهر التراكيز المخففة لمستخلص الثوم فعالية تثبيطية تجاه هذه البكتريا, ومن نتائج الدراسة ان عزلتين (6.6%) من بكتريا العنقوديات الذهبية كانت مقاومة لجميع التراكيز. أظهر التركيز 3.2ملغم/مل لمستخلص الثوم المثبط والقاتل الادنى ولجميع عزلات الدراسة. نستنتج من خلال هذه الدراسة ان التركيز العالي لمستخلص الثوم له فعالية تثبيطية عالية تجاه بكتريا العنقوديات الذهبية *Staphylococcus aureus* . هناك حاجة لدراسة الفعالية التثبيطية لمستخلص الثوم داخل الجسم الحي .