

ANTIBACTERIAL PROPERTIES OF AQUEOUS GARLIC EXTRACT AGAINST SOME PATHOGENIC BACTERIA IN VITRO.

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ABSTRACT

The antibacterial effects of crust removed aqueous garlic extract using diethyl –ether against resistant pathogenic bacteria G+&G-isolates including Enteropathogenic *E.coli* 0.127, *Bacillus cerues* ,*Listeria monocytogenes* *Salmonella typhi* and *Pseudomonas aeruginosa* ,were studied .The first concentration don't give any result(0.25,0.50.0.75) therefore increasing the concentration. Antibacterial activity by well-diffusion and used different concentration 0.125% ,0.150% , 0.175 % .The result of inhibition zones of *L . monocytogenic* (33mm – 37mm) for gram positive while gram negative such as *salmonella typhi* don't effect to the garlic extract.

Key words: Garlic, Allicin, Antibacterial activity.

INTRODUCTION

Garlic is one of the edible plants, which has generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms including, bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Moreover, garlic has been reported to reduce blood lipids and anticancer effects. Chemical analyses of garlic cloves have revealed unusual concentration of sulfur-containing compounds (1—3%) (Yeh and Liu , 2001.The compound turned out to be an oxygenated sulfur compound which they termed allicin,from the Latin name of the garlic plant,(*Allium sativum*).Pure allicin is a volatile molecule that is poorly miscible inaqueous solutions and which has the typical odor of freshly crushed garlic (Yin *et al* .,2002) proof of the chemical structure of allicin in 1947, when it was shown that allicin could be synthesized by mild oxidation of diallyl disulfide(Okochi *et al.* , 2000) The debate on the presence of allicin in crushed cloves versus its absence in odorless intact cloves was resolved after it was isolated (Akinyemi, 2000), identified, and synthesized an oxygenated sulfuramino acid that is present in large quantities in garlic cloves and which they named alliin. Alliin was found to be the stable precursor that is converted

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to allicin by the action of an enzyme termed alliinase which is also present in the cloves(Tsao ,2001).

Aim of the study

Isolate and diagnose some types of pathogenic bacteria from various sources using the Vitek compact .The effect of the active ingredient of garlic (allicin) against pathogenic bacteria used in the study.

MATERIALS AND METHODS

Sources of test organisms and garlic:

The microorganisms used for this study were isolated from stool such as *Salmonella typhi* (Cowan , 1974) and *Enteropathogenic E.coli 0.127* (McNeish, 1992). *Listeria monocytogenes* from Cheeses (Beuchat and Ryu, 1997). *Bacillus cereus* from Of rice (Nygren, 1962), both of which caused diarrhea due to food poisoning. *Pseudomonas aeruginosa* from Infected wounds(Cowan, 1974).Occurent out food technical biology center / Microbiology Lab.

Microbiological analysis of samples

Samples were inoculated on tryptone soya broth (Oxoid, England), a general purpose broth that can support the growth of both aerobes and anaerobes.MacConkey agar used for the isolation of *Enteropathgenic E.coli 0.127*-like enteric organisms and confirmation by PCR (Higuchi and Dollinger , 1992), Xylois lysine agar for isolation of *Salmonella typhi* and confirmation by Antisera somatic O . *Pseudomonas* agar used for isolation of *P.aeruginosa* and confirmed by blue pigment . Frezer broth with supplement half frezer used for isolation *L.monocytogenes* . *Bacillus* selective agar for the isolation of *Bacillus cereus* and incubated at 37°C for 24 hours. Primary isolates were repeatedly subcultured on fresh media using plate streaking techniques to obtain pure cultures. All strains are confirmed by Vitek compact-2 (biomerix). The purified isolates were stored in agar slant at 4°C for later characterization and identification.

Preparation of garlic extract

Mixing 50 grams of garlic crust removed with 200 ml of distilled water and then nominated tow phases. The first raw canvas and the second of the nomination paper .Blending the filtrate with amount equal to the compound Diethyl ether in separating funnel for the purpose of Purifying the filtrate from

fatty oils done remove the bottom layer of the mixture and steamed in a rotary evaporator and the temperature of 50 C for the purpose of the filtrate purification filtrate from residues Diethylether .Filtrate stored in opaque botteles to use(Bonle and Desmukh, 1975).

Antibacterial effects of raw garlic extract

Hole diffusion technique using the stuck for each bacterial isolate by measured by McFarland device and planned to center muller Hintomarked.0.125,0.150.0.175 Mm of garlic extractions were added on surface center of bacteria medium used in the study.

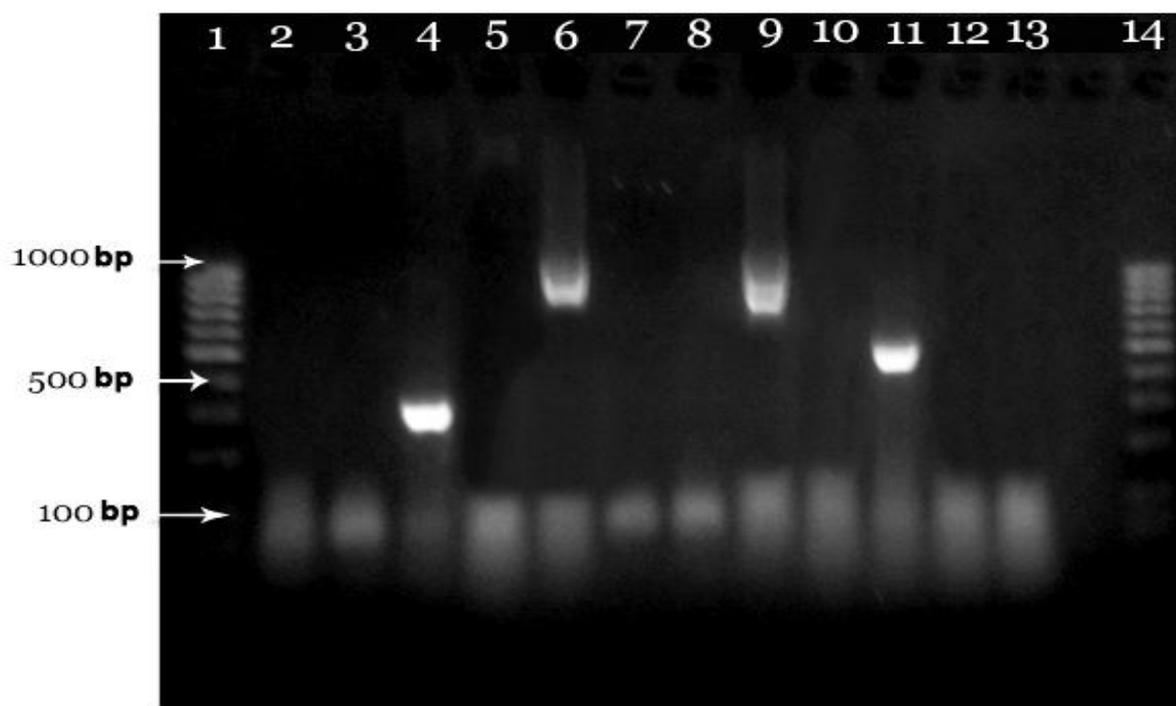


Figure 1. Gel electrophoresis of amplified *bfpA* (326bp), SLT I (894bp) and SLT II (478bp) from *E. coli* DNA isolated from stool specimens. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidium bromide and visualized on a UV transilluminator.

Lane 1 and 14 . 100 bp DNA ladder.

Lane 2-5. Amplified *bfp* gene of Serogroups 55, 86, 127 and control respectively.

RESULTS AND DISCUSSION

Table 1: Sensitivity test for Garlic extract by Diethyl ether as antimicrobial for some Pathogenic bacteria as MIC (minimum inhibitory concentration) .

contro 1	<i>L.monocytogenes</i> (mm)	Pathogenic <i>E.coli</i> 0.127 (mm)	<i>B.cereus</i> (mm)	<i>P.aeruginos</i> <i>a</i> (mm)	<i>S.typhi</i> (mm)	Concentra- tion of garlic extract (mm)
Zero	-	-	-	-	-	0.25
Zero	-	-	-	-	-	0.50
Zero	-	-	-	-	-	0.75

Table2: Sensitivity test for Garlic extract by Diethylether as antimicrobial for somePathogenic bacteria as MIC (minimum inhibitory concentration)

control	<i>L.monocytogenes</i> (mm)	Pathogenic <i>E.coli</i> 0.127 (mm)	<i>B.cereus</i> (mm)	<i>P.aeruginos</i> <i>a</i> (mm)	<i>S.typhi</i> (mm)	Concentra- tion of garlic extract (mm)
Zero	33	11	17	-	-	0.125
Zero	37	13	19	-	-	0.150
Zero	37	17	19	-	-	0.175

Show Table1:the garlic extraction for first concentration didn't give any result therefore the concentration increasing.

Bacterial isolates from the different samples were principally *Enteropathogenic coli* 0.127 ,*B. cereus*,and *L.monocytogenes*, were used for sensitivity test with garlic concentration table 2, which shows the zones of inhibition of bacterial growth by garlic,*L.monocytogenes* was highest sensitivity to garlic extract, *B.cereus* was least sensitive against garlic extract ,and the Enteropathgenic *E.coli* 0.127 Less sensitivity. While *P.aeruginosa* and *S.typhi* not affected garlic extract all the concentrations used in the study. the active ingredient of garlic the compound turned out to be an oxygenated sulfur molecule which they termed(Allicin), (Hughes and Lawson, 1991), acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Feldberg *et al*, 1988). and thus interferes with DNA transcription and other activities involving DNA (Prescott *et al*, 2005). In this study gram-negative bacterial isolates were less sensitive to garlic than gram positive. The cell wall structural nature of gram-negative enteric bacteria may be responsible for the susceptibility resisting. For instance the bactericidal effect of garlic might be due to the structural characteristics of organismswhich

play a role in the bacterial susceptibility to garlic constituents (Tynecka and Gos, 1975), particularly Gram positive contains only 2% lipid (Salton, 1964) so that the lipid content of the membranes will have an effect on the permeability of allicin and other garlic constituents. Hence these phenomenons may favor the destruction of the cell wall and genetic materials of gram positive The lipid composition of cell wall has been found to have an influence on the permeability of hydrophobic and volatile bioactive substances in garlic (White , 1998) whereas the cell wall of gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid (Carpenter, 1968). In a study of (Sivam, 1998) in which he The cell membrane of *E. coli* has been reported to contain 20% lipid . The polysaccharides and the lipid contents of the cell wall affect of very high the permeability of allicin and other garlic constituents, the effect of garlic extract is most pronounced on enteric bacterial pathogens. The absence of resistance to garlic enhances its ability to effectively act against even highly resistant bacterial isolates, such as *Pseudomonas aeruginosa*. It therefore appears attractive that garlic affect DNA and RNA syntheses . In another study this may be responsible for the difference in susceptibility to garlic between gram negative *Helicobacter pylori* and gram-positive *Staphylococcus aureus* (Cellini *et al.*, 1996) .

CONCLUSION

Garlic (*A. sativum*) has antimicrobial properties against gram positive It has both a bacteriostatic and bactericidal activity when tested *in vitro* using crude preparation of garlic. Therefore, garlic may be used successfully for treating food poisoning causative agent like gram positive further *in vivo* studies are necessary.

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خواص المضاد المايكروبي للمستخلص المائي للثوم ضد بعض انواع البكتريا الممرضة خارج الجسم الحي

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المستخلص

تم دراسة تأثير المستخلص المائي للثوم المزال منه القشره باستخدام داي اثيل ايثر كمضاد للبكتريا السالبة والموجبه لصبغة كرام ضد خمسة عزلات مرضية مقاومة للمضادات البكتيرية وهي *Salmonella typhi*، *Listeria monocytogenes*، *Bacillus cereus*، *E.coli* 0.127 و *Pseudomonas aeruginosa* قيد الدراسة . في البداية استخدمت تراكيز غير مؤثره 0.25% و 0.75%، 0.50%، ولم تعطي أي نتائج، فتم زيادة التراكيز وظهرت النتائج نشاط المستخلص ضد البكتريا الموجبة والسالبة لصبغة كرام باستخدام الانتشار في الحفر حيث استخدمت تراكيز مختلفه 0.25%، 0.150، 0.175% وكانت نتائج التثبيط للبكتريا الموجبة *Listeria monocytogenes* (33ملم - 37ملم) بينما البكتريا السالبة مثل السالمونيلا لم تتأثر بمستخلص الثوم. تم الكشف عن العزلات البكتيرية باستخدام الفحوصات المورفولوجيه والكيموحيويه مثل API 20 E والفحوصات الجزيئية (PCR) .

الكلمات المفتاحية: الثوم ، الالسين ، فعالية المضاد الحيوي .