ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Inhibitory Effects of Aqueous Garlic Extract (AGE) against multidrug resistant from *Staphylococcus aureus* and *Escherichia coli.*

[1]Aqeel Ismael Gheni , [2]Yaseen Noori M. AL-Shekhany & [2]Harem Othman Smail

1. Dept. of Medical Microbiology / Faculty of Science & Health /University of koya.
2. Dept. of biology / Faculty of Science & Health /University of koya.

1&2 Koya University. University Park, Danielle Mitterrand Boulevard, Koysinjaq KOY45, Erbil, Iraq.

Manuscript Info

Manuscript History:

Received: 14 November 2015
Final Accepted: 22 December 2015
Published Online: January 2016

Key words:

Corresponding Author

Aqeel Ismael Gheni.

Abstract

This study was conducted to investigate the potential of Aqueous Garlic Extract (AGE) as an antimicrobial agent instead of antibiotics through evaluation susceptibility of aqueous extract on pathogen bacteria and determination of values minimum inhibitory concentration (MIC). Has been chosen of 24 isolates of different human infections included (14) Gram positive bacteria (*Staphylococcus aureus*) and (10) Gram negative bacteria (*E. coli*) which were resistant at least for five types of antibiotic discs through evaluate susceptibility by Kirby-Bauer method according to NCCLS guidelines whereas the sensitivity of tested bacteria against aqueous garlic extract were evaluated through hole plate diffusion method. The MIC of aqueous garlic extract were determined by two-fold serial dilution with concentrations ranging from 500 mg $ml^{\bullet 1}$to 3.91 mg $ml^{\bullet 1}$. (57.14%) *Staphylococcus aureus* isolates can't grow in concentration 31.25 mg $ml^{\bullet 1}$ (susceptibility), while (70 %) from *E. coli* isolates can grow in this concentrations of extract (resistance), so the MIC values for *A. sativum* were ranged from 62.5 mg $ml^{\bullet 1}$ to 31.25 mg $ml^{\bullet 1}$ .As important conclusion, the Garlic (*Allium sativum*) extract had bactericidal and bacteriostatic effects, especially on isolates of *Staphylococcus aureus* & *E. coli*, these isolates shown the most affected by the Garlic extract at all concentrations except low concentrations and Garlic extract was able to use effectively for controlling the bacterial growth.

Introduction:-

The increasingly high numbers of bacteria that are developing resistance to classical antibiotics **(1,2)** drive much of the current interest on plant antimicrobial molecules in hope that they may provide useful leads into anti-infective drug candidates. Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and threnodies amongst which can be cited xanthones, benzophenones, coumarins and flavonoids **(3, 4).**

Garlic, *Allium sativium* is a member of the Alliacea family , has been widely recognized as valuable spice and a popular remedy for various bacterial infections .The name of garlic may have originated from the celtic word all meaning pungent .Cultivated practically throughout the world ,garlic appear to have originated in central Asia and spread to China ,the near east and the Mediterrnean region before moving west to central and southern Europe ,Northern Africa and Mexico **(5)** .Recently reviewed the therapeutic effect of garlic namely , effects of cardiovascular system , antibiotic and anti-inflammatory effects **(6)** .

The principal antimicrobial compounds of *Allium is* (Aqueous garlic) belonging to a group known as thiosulfate. The antimicrobial activity of thiosulfates has been explained as a general reaction between thiosulfates and -SH

groups of essential cellular proteins. Mentioned that -S (O) S- was responsible for the antimicrobial activity and that reacted readily with cysteine to yield mixed disulfides. The general reaction, can apply to where thiosulfates are involved and the reaction is believed to be the common mechanism of antimicrobial activity of thiosulfates. The general inhibitory mechanism of thiosulfates, are belong to specific target processes or enzymes of thiosulfates. The thiosufinates responsible for inhibition of the bacterial growth (allinase) (**7**). In addition, allicin, one of the sulfur-compounds responsible for garlic's characteristic odor, is a powerful antibacterial and antiviral agent that joins forces with vitamin C to help kill harmful microbes. In research studies, allicin has been shown to be effective not only against common infection like colds, flu, stomach viruses, and Candida yeast, but also against powerful pathogenic microbes including tuberculosis and botulism (**8**).

Various garlic preparations have been shown to exhibit a wide of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus,* and *Clostridium.* Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to aqueous garlic. Aqueous garlic extracts are also effective against *Helicobacter pylori* the cause of gastric ulcer (**9**).Various bacterial strains resistant to antibiotics such as *Staphylococcus aureus* & *E.coli.* Aqueous garlic has an important role in the cure of much bacterial infection, because most of the pathogenic bacteria are sensitive for the aqueous garlic. The activity of aqueous garlic against bacteria belong to the allicin .Allicin is substance found in the aqueous garlic and responsible for the inhibition of toxins of the bacteria (**10**). *S. aureus are* multi-drug resistant (resistant to Ampicilin, Amoxilin, Navobiocin, Chloramphenicol, Optochin, Piperacillin, Nalidixic Acid, Ceftriaxon ,Amikacin ,karomycin)(**11**). Another widely studied garlic preparation is aged garlic extract (AGE). Garlic is one of the most investigated medicinal plants. During 1960 to 2007, more than three thousand research papers have been published on the chemistry and biological effects of garlic and garlic preparations (**12**).

Materials and methods:-

Garlic and aqueous extract preparation:-

Allium sativum or garlic bulb was bought from local market in koya, Erbil. The sample was crush into small pieces and exposed overnight to *UV* light in the laminar flow. The sample was then blended with sterile water at concentration of 1g/ml. The blended sample was filtered through muslin cloth and the extract was kept at 4°C until used. Antibiotic disks that applied in this study were 10 different types (Bioanalyse®) for screening test **(13)**.

Bacterial isolates:-

The study includes examining (29) isolates from *Staphylococcus aureus* and (27) isolates from *E.coli*, selects (24) isolates were resistance at least 5 types from antibiotics according to antimicrobial susceptibility test (**14**) (where using 10 types from antibiotic disks) show table 1 & 2.

The isolates were obtained from the Microbiology Department, Faculty of science and health, University of Koya .The isolates were identified as *Staphylococcus aureus* using standard procedures .All the isolates were clinical strains recovered from patients with human infection. Antibiogram profiles of the organisms by the disk diffusion method revealed to at least three antibiotics based on national committee for clinical Laboratory standards, isolates were maintained on tryptic soy agar slants at 4 ^{o}C prior to use.

Antibiogram Activity:-

The antibiogram assay was carried out using well diffusion method against Gram positive and negative cultures. The inoculums were grown in nutrient broth overnight and about (0.1 ml) of test bacterial cultures were spread over on Muller - Hinton agar medium (pH 7.5 at 25 ºC) in sterile peteri plates. Circular wells (6 mm) were cut in the agar culture media and filled with 100 µL. For negative control, disks were impregnated with sterile water. For positive control different antibiotic disks were used. Plates were incubated at 37 C for 48 h and diameters inhibition zones (mm) were determined.

Determination of MIC value:-

Minimum Inhibitory Concentration (MIC) of *A. sativum* extract against the tested bacteria was determined using two fold dilution method in microtiter plate. The concentration of the *A. sativum* extract were ranged from 500 mg ml^{-1} to 3.91mg ml^{-1}. Each assay was run in triplicates. The inoculated plates were incubated for 37 °C for 24 hours. After incubation period, the MIC values were determined by observed the turbidity of the wells in the microtiter plate. Well of the microtiter plate that showed no turbidity was interpreted as no growth of the tested bacteria. The MIC was defined as the lowest concentration of *A. sativum* extract or antibiotics that can inhibit the growth of the tested bacterial.

Results and discussion:-

Table 1-2 shows the resistance (24) of the bacterial isolates at least (5) of antibiotics according to antimicrobial susceptibility test (**14**) (where using 10 types from antibiotic disks).

Table1: Resistant patterns of (14) Multidrug resistance (MDR) strains of *Staphylococcus aureus* recovered from UTI to antimicrobial agent disk diffusion test.

Pattern number	Resistance patterns	Number of isolates
1	AM,AX,NV,C,OP,PRL	5
2	AM,C,OP,NA,AX, CEF	3
3	AM,NV,OP,AK,K	6
		14 isolates

Table-2-Resistant patterns of (10) Multidrug resistance (MDR) strains of *E. coli* recovered from UTI to antimicrobial agent disk diffusion test.

Pattern number	Resistance patterns	Number of isolates
1	AM,AX,NV,C,OP,PRL	5
2	AM,C ,NA,AX, K	2
3	AM,NV,CEF,OP,AK	3
3 Patterns		10 isolates

(1) AM-Ampicilin, (2) AX-Amoxilin, (3) NV-Navobiocin (4) C-Chloramphenicol (5) OP-Optochi (6) PRL-Piperacillin (7) NA-Nalidixic Acid (8) CEF-Ceftriaxon (9) AK-Amikacin (10) K-kanomycin.

The diameter of the zones of inhibition around the wells varied with ranged (9 -28 mm) for S. aureus isolates while E. coli isolates were less and ranged (8-19mm) indicating that all (24) isolates (100%) were sensitive to aqueous garlic extract, show table 3 & 4.

Table-3- Inhibitory effect of Garlic extract on the (14) MDR strains of *Staphylococcus aureus* by disk diffusion test.

Range of zone inhibition mm	Number of isolates	%
9 - 12	3	% 21.4
14 -18	6	% 42.9
20 -24	3	% 21.4
26 -28	2	% 14.3
	14 isolates	% 100

Table-4- Inhibitory effect of Garlic extract on the (10) MDR strains of *E. coli* by disk diffusion test.

Range of zone inhibition mm	Number of isolates	%
8 - 10	4	% 40
12 - 15	4	% 40
17 -19	2	% 20
	10 isolates	% 100

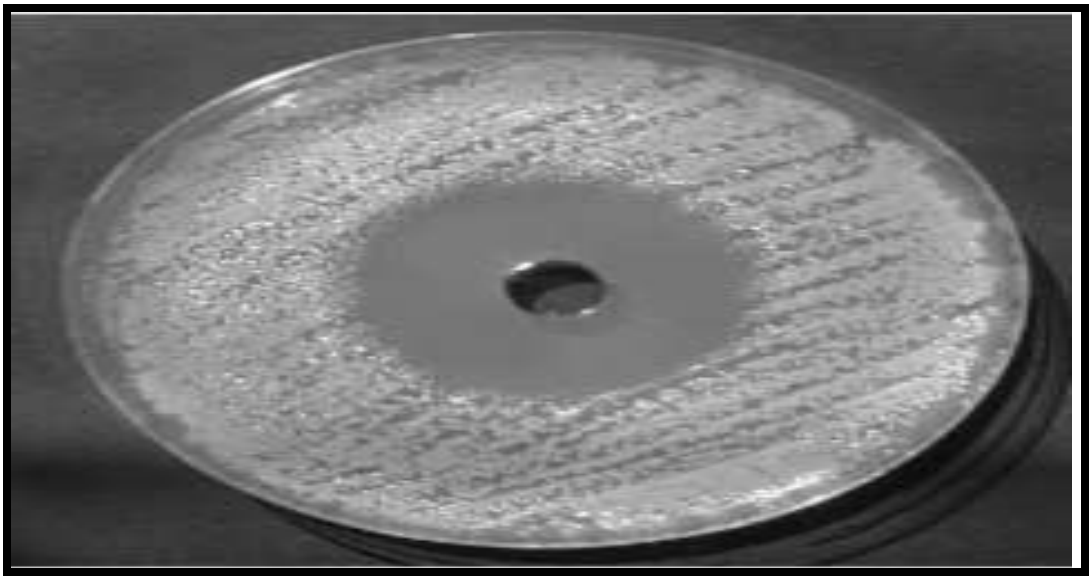

Figure 1: Show zone of inhibition produced by aqueous garlic extract

Table 5: Minimum inhibitory concentration of *Allium sativum* extract against the tested bacteria

concentration (mg ml^{-1}) / Number of isolates	500	250	125	62.5	31.25	15.63	7.81	3.91
9 Sa	-	-	-	-	-	+	+	+
5 Sa	-	-	-	-	+	+	+	+
7 EC	-	-	-	-	+	+	+	+
3 EC	-	-	-	-	-	+	+	+
24 isolates								

- Absence of growth, + presence of growth, Sa = *Staphylococcus aureus* & EC= *E. coli*

Had been chosen (24) of out (56) isolate for this study which were resistance for five different types of antibiotics at least, according to method of Kirby-Bauer show Table (1 and 2) which has included (10) isolate from *E. coli* and (14) from *Staphylococcus aureus*, (**15**). The basis of the use of aqueous extract of garlic has been strong antibacterial agent and acts as an inhibitor on both gram positive and gram negative such species as *Escherichia coli, Salmonella, Streptococcus, Staphylococcus, Klebsiella, Proteus mirabilis and Helicobacter pylori* (**7**; **16**; **17**). So it been chosen both *S.aureus* and *E. coli* which have medical important because many infection cause by this bacteria such diarrhea, UTI, skin infections , meningitides and food poisoning (**18**), also toxins and extracellular enzymes

produce by *S. aureus* include enterotoxin, hemolysins, DNAase, lipase, coagulase.. etc. (**15**). The bacterial growth inhibition zones were observed and measured. The size of the growth inhibition zones would indicate the effectiveness of the Garlic extracts in controlling the bacterial infection, in our study the zones of inhibition had been different which ranged (9 -28mm) in *S. aureus* while *E. coli* were been less ranged (8-19mm) this is consistent with the study **9** also **10** who noted various isolates resistant to antibiotics and sensitivity to aqueous garlic extract.

Table 5 were showed Minimum Inhibitory of AGE against tested bacteria in present study, The MIC values of AGE was ranged from 62.5 mg ml•^{1}to 3.91 mg ml•^{1} (**19**) Claimed that generally plant extract was more effective in term of inhibit the growth of Gram positive bacteria than Gram negative bacteria, has been showed our study that the MIC value 31.25 mg ml•^{1} reached (57.14%) to *S. aureus* while reached (30%) of *E. coli* isolates, This is consistent with a study which has been carried by (**20**) which has shown that value 31.25 mg ml•^{1} for AGE was very significant against *S. aureus* and *E. coli*. had noted at study (**21**) that garlic has a bactericidal effect at the lower concentration of 15.25 mg ml•1 for clinical isolate of *S. aureus* while our study had been explained that the tested isolates were not grow in this concentration. The cell membrane of *E. coli* has been reported to contain 20% lipid (**22**). The polysaccharides and the lipid contents of the cell wall affect the permeability of allicin and other garlic constituents, and thus the observed susceptibility to garlic by the diarrheagenic organisms (**23, 21**). In the present study well diffusion method of garlic extract showed various difference pattern or size, this findings indicated that crud extract possess antibiogram activity significantly for both Gram positive as well as Gram negative bacterial cultures, which also showed that variation in diameter is mainly due to the cell wall of peptidoglycan and lipopolysaccharide content of bacterial of bacterial cell wall. Allicin is obtained by crushing or cutting garlic cloves Allicin acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin (**24**). Antimicrobial activity of garlic could be explained by blocking mechanism by which allicin blocks certain groups of enzymes as cysteine proteinases and alcohol dehydrogenases.(**25)** These groups of enzymes are found in a wide variety of infectious organisms such as bacteria, fungi and viruses and this provides a scientific basis for broad-spectrum antimicrobial activity of garlic. The mechanism responsible for all these activities is believed to be allicin and its chemical reaction with thiol groups of various enzymes (**26**). It is of interest that cooked garlic and commercially available garlic tablets lost the antibacterial effect found in raw garlic. As medicinal chemists advance in their search for new bacterial targets to attack, bacteria relentlessly evolve; as a result, a large number of bacterial species have become resistant to antibacterial drugs (**27, 28**). This might be due to the garlic species variation in different country, the processing difference on the garlic species and the inoculums densities. Hence these phenomenons may favor the destruction of the cell wall and genetic materials of *Staphylococcus aureus* lastingly **(29).**

References:-

1. Kiessling J., S. Kruse, S.A. Rensing, K. Harter, E.L. Decker, and R. Reski. 2000. Visualization of a cytoskeleton-like FtsZ network in chloroplasts. J. Cell Biol. 151:945-950.
2. Cole, L., Bardgett, R.D., Ineson, P. and Hobbs, P.J. 2002. Enchytraeid worm (Oligochaeta) influences on microbial community structure, nutrient dynamics and plant growth in blanket peat subjected to warming. *Soil Biology & Biochemistry* **34**: 83-92.
3. Nkengfack, A.K., Mkounga, P., Fomum, Z.T., Meyer, M. & Bode, B.,(2002). Globulixanthones C, D and E; three prenylated xanthones with antimicrobial properties from the root bark of Symphonia globulifera. Phytochemistry 51:181- 187.
4. Komguem, J., Meli, A.L., Manfouo, R.N., Lontsi, D., Ngounou, F.N., Kuete, V., Kamdem, H.W., Tone, P., Ngadjui, B. T., Sondengam, B.L. and Connolly, J.D. (2005) Xanthones from Garcinia smeathmanni (Oliver) and their antimicrobial activity. Photochemistry 66, 1713-1717.
5. Shokrazadeh and Ebadi; (2004).Antibacterial effect of garlic (Allium sativium) on Staphylococcus aureus.;Department of Toxicology ,Faculty of pharmacy, Mazandaran University of Medical science ,pp:11.
6. Srinivasan, (2009). P. Lakshmanaperumalsamy; In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature, Electronic Journal of Biology, pp: 5-10 .
7. Ankri S, Mirelman D: Antimicrobial properties of allicin from garlic. *Microbes Infect 1999;* **1(2):** 125-9.
8. Shobana,S., Vidhya ,V.G and and Ramya, M. (2009) Antibacterial Activity of Garlic Varieties (Ophioscordon and Sativum) on Enteric Pathogens Curr. Res. J. Biol. Sci., 1(3): 123-126.
9. Ghannoum, M. A. (1988). Studies of the antimicrobial mode of action of Allium sativum (garlic)J. Gen. Microbiol., pp:134.
10. Shin, D.B., Kim, Y.S. and Lee, Y.C. (1999). Effect of dehydration methods on the antimicrobial activity of garlic juice powder, 1999 IFT Annual Meeting, pp: 98.

11. Ashley Hagler; (2007). Antibiotic Resistance Ninecia Scott Topics in Medicine and Biology Summer Ventures, pp: 155-156.
12. Vinay K Singh, Dinesh K Singh ;(2008). Pharmacological Effects of Garlic (*Allium sativum L.*) Department of Zoology, Gorakhpur University, pp: 3.
13. Elsom, Giles K., Denis Hide and David M. Salmon.(2000) An antibacterial assay of aqueous extract of garlic against anaerobic microaerophilic and aerobic bacteria. Microbial Ecology in Health and Disease . 12: 81–84.
14. National Committee for Clinical Laboratory Standards. (2000). Performance standards for antimicrobial disk susceptibility test, 7th edition. Approved standards, NCCLS Document M2- A7, Vol; 20 no 1; Wayne PA. USA.
15. Muhsin , A. Jabar and Amina Al-Mossawi ; (2007).Susceptibility of some multiple resistant bacteria to garlic extract Microbiology Department ;pp:98-99.
16. Reuter, H. D., H. P. Koch and D. L. Lawson. 1996. Therapeutic effects and applications of garlic and its preparations. In: H. P. Koch and L. D. rich plant antifungal proteins from Brassicaceae species. FEBS Lett. 316:233–40.
17. Ducel G, Fabri J, WHO (2002). In: Prevention of hospital aquired infections: epidemiology, of nosocomial infections. A Practical guide. 2nd edn. Nicolle L (eds). Malta, pp: 4.
18. Lin, J., A.R. Opaku, M. Geheeb-Keller, A.D. Hutchings, S.E. Terblanche and A.K. Jager, (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *J. Ethnopharmacology*, pp: 37.
19. Sivam, G.P., J.W. Lampe, B. Ulness, S.R. Swanzy and J.D. Potter, (1997). Helicobacter pylori in vitro susceptibility to garlic (Allium sativum) extract. Nutr. Cancer, pp: 27.
20. Sivam, GP. Protection against *Helicobacter pylori* and other bacterial infections by garlic (Abstract). Newport Beach CA: The Conference of the Recent Advances in the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. 15-17 November 1998.
21. Cellini L, DiCampli E, Masuli M, DiBartolomeo S, Allocati N. Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). *FEMS Immunol Med Microbiol* 1996; 13: 273-7.
22. Feldberg R.S., Chang S.C., Kotik A.N., et al. (1988) In vitro mechanism of inhibition of bacterial growth by allicin. *Antimicrob Agents Chemother.,* 32: 1763-1768.
23. Iwalokun BA, Ogunledun A, Ogbolu DO,Bamiro SB, and Jimi-Omojola J; (2004). In Vitro Antimicrobial Properties of Aqueous Garlic Extract Against Multidrug-Resistant Bacteria and Candida Species from Nigeria. J Med Food 7 (3) 2004, 327 Ð333.
24. Rabinkov, A., T. Miron, L. Konsrantinovski, M. Wilchek, D. Mirelman and L. Weiner 1998. The mode of action of allicin: trapping of radicais and interaction with thiol containing proteins. Biochim. Biophys. Acta 1379:233-244.
25. Gould, I.M., (1994). Risk factors for acquisition of multidrug-resistant gramnegative bacteria. Eur. J. Clin.Micrbiol. Infect. Dis., 13(suppl.): pp: 38.
26. Sanders, C.C. and W.E.Jr. Sanders, (1992). B-Lactam resistance in gramnegative bacteria: global trends and clinical impact. Clin. Infect. Dis., PP:15.
27. Acheampong YB, El-Mahmood AM, Olurinola PF (1988). Antibacterial properties of the liquid antiseptic TCP. Indian J. Pharm, *pp*: 183-86.
28. Cuviello, P.V., (1999). The many faces of Staphylococcus.J. Am. Med. Technol., pp: 1.
29. Edward Alcamo ;(2005).Deadly disease and epidemics Staphylococcus aureus infection, Distinguished Teaching Professor of Microbiology,SUNY Farmingdale , pp:75.