International Journal of Current Research in Life Sciences
Vol. 06, No. 01, pp. 637-640, January- 2017

www.ijcrls.com

Full Length Research Article

INHIBITORY EFFECT OF AQUEOUS GARLIC EXTRACT (*ALLIUM SATIVUM*) ON DRUG RESISTANT BACTERIAL ISOLATES

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Accepted 17th December, 2016; Published Online 31st January, 2017

ABSTRACT

An inhibitory effect of aqueous garlic extract on 12 drug resistant clinical isolates *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth was evaluated. The antimicrobial effect of aqueous garlic extract in different concentrations 60%, 50%, 40%, 30%, 20% and 10% was examined by well diffusion method and the antimicrobial sensitivity test for selected group of antimicrobial agents was examined by disc diffusion method. The results revealed that antibacterial activity of aqueous garlic extract at60%, 50% and 40% concentration characterized by clear inhibition effect on *Staphylococcus aureus* growth with range of diameter of inhibition zone was 14 mm. For *Pseudomonas aeruginosa*, high concentration of 60% gave an inhibition zone of 10 mm, while 50%, 40% and 30% concentrations gave a diameter of 9 mm. The results of antimicrobial sensitivity test revealed that 50% of isolates were sensitive to ciprofloxacin, whereas the resistance of the isolates for cefuroxime sodium, cefotaxime, amoxicillin/clavulanic acid and lincomycin were 100%, 92%, 83% and 67% respectively. The study concluded that the resistant and multiple antimicrobial agents resistant were common in the isolates, whereas all isolates tested were sensitive to aqueous garlic extract that could be used as an antibiotic for treatment of bacterial infections.

Key words: Inhibitory Effect, Garlic, Drug resistant, Bacterial Isolates.

INTRODUCTION

Staphylococcus aureus and *Pseudomonas aeruginosa* are clinically pathogenic bacteria (Bosso, 2005; Hauser and Sriram, 2005), with the increased medical use of antibiotics some of such strains had shown high resistance and multi-resistance for antibiotics which makes the treatment with such traditional antibiotics very hard to recover from the infection of such pathogens (Maltezou and Giamarellou 2006 ; Obritsch *et al.,*2005). Garlic is considered a bulbous plant in which the species (*Allium sativum*) belongs to Liliaceaefamily, and it has a nutritional and medicinal properties (Bakri and Douglas, 2005 ;Ross*et al.,* 2001), in which it has anti-microbes effect to inhibit the growth of the Gram positive and Gram negative bacteria (Abouelfetouh and Moussa, 2012) as well as the fungi andyeasts (Lemar *et al.,* 2005 ; Shams-Ghahfarokhi *et al.,* 2006), parasites and viruses (Ayaz and Alpooy, 2007), furthermore, garlic has a potent effect to minimize the risk of gastric ulcers and cancer due to its inhibitory effect on *Helicobacter pylori*in particular for those people who eat garlic on large quantities as such a pathogen has a correlation with stomach cancer (Brown *et al.,* 2009; Sivam, 2001). Garlic has biological effects and properties such as antimicrobial, anti-inflammatory, anticancer, anti-thrombotic and anti-atherosclerotic (Yutani, 2011), also it has protective properties such as a protection against cardiovascular diseases, the blood pressure balance, reduction of glucose and blood cholesterol, enhancement of immune system, in addition to antioxidant properties (Ayazand Alpooy, 2007). The antimicrobial effectof garlic is due to the presence of allicin and if such substance was completely removed, the garlic will have no effect against microbes (Cai, 2007), and the amino acid (allins) in the garlic is treated by metabolism via allinase enzyme which named (cysteine sufoxidelyase) into allicin and other thiosulfinates compounds (Ayazand Alpooy, 2007). Allicin's main acts to inhibit the synthesis of RNA completely while it inhibits the synthesis of DNA partially, so it is proposed that RNA is the main target for allicin (Eja *et al.,* 2007). The study aims at determiningthe inhibitory effect of the aqueous garlic extract and selected group of antimicrobial agents that has similar inhibitory effect of the garlic against the growth of drug resistant clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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MATERIALS AND METHODS

Sources of bacterial isolates and garlic

A total number of 12 clinical bacterial isolates including 6 isolates for the *Staphylococcus aureus* and other 6 isolates for the *Pseudomonas aeruginosa* that were isolated and diagnosed at the laboratory of medical microbiologyin the national center of the central public health laboratories –Hadhramout/Yemen.

The isolates were preserved in brain-heart infusion broth (Oxoid) with 10% glycerol at a temperature of -4C. The garlic (*Allium sativum*) was chosen in this study which is highly available in the local markets.

Preparation of the aqueous garlic extract

Garlic cloves were peeled, well-chopped and mixed in a blender, then they were purified via a medical gauze to obtained the raw garlic extract of different concentrations 60%, 50%, 40%, 30%, 20% and 10%that prepared by mixed garlic cloves of 15 grams, 12.5 grams, 10 grams, 7.5 grams, 5 grams, 2.5 grams for each concentration respectively to 25ml of sterile distilled water. The extract of the concentrations were preserved in sterile glass bottles in the refrigerator until to be used.

All plates of the tested organisms was then allowed to incubate at 37°C for overnight. The diameters of the zone of inhibitions were measured by measuring scale in millimeter.

Antimicrobial susceptibility test

A disc diffusion technique using the Kirby-Bauer method was applied in testing pure cultures of the isolates for their antimicrobial sensitivities. Muller-Hinton agar (Oxoid) was used to measure the affectivity of the antimicrobial agents, cefuroxime sodium (30µg), amoxicillin/clavulanic acid (30µg), cefotaxime (30µg), amikacin (30µg), ciprofloxacin (5µg) and lincomycin (15µg) according to the clinical and laboratory standards institute procedures (CLSI, 2012).

Table 1. The inhibitory effect of the aqueous garlic extract on bacterial isolates by agar wells diffusion method

Bacterial isolates	Ratesof inhibitory zones in millimeter for aqueous garlic extract concentrations					
	60%	50%	40%	30%	20%	10%
Staphylococcus aureus	14	14	14	11	11	8
Pseudomonas aeruginosa	10	9	9	9	9	6

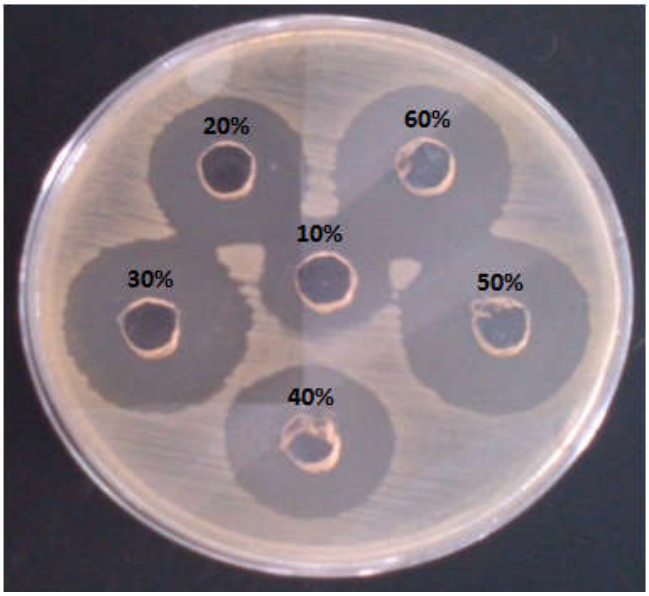

Fig. 1. The inhibitory effect of the aqueous garlic extract on bacterial isolates by agar wells diffusion method

Testing for the antibacterial effects of aqueous garlic extract by wells diffusion method

Loop full growths from bacterial isolates were subculture into nutrient agar (Oxoid) and incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline, adjust the turbidity and compared to 0.5 of McFarland standard to yield a uniform suspension an approximate number of the cells which is equal to 10^8 CFU/ml. Muller-Hinton agar (Oxoid) was streaked with the bacterial suspension using a sterile cotton swab, then the plates were left to dry for 15 minutes at room temperature (NCCLS, 2012). Agar wells diffusion method as described by (Cutler and Wilson, 2004) with minor modifications was used. Wells with a diameter of 8mm were cut in the Muller-Hinton agar using a sterile cork borer as per the required concentrations and filled with 100µl of aqueous garlic extract.

RESULTS

In this study, the results of the aqueous garlic extract had shown an inhibitory effect of the garlic for all bacterial isolates, table (1) and figure (1).

Table 2. Results of sensitivity test of bacterial isolatesby disc diffusion method

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Cefuroxime sodium	0	0%	0	0%	12	100%
Cefotaxime	0	0%	1	8%	11	92%
Amoxicillin/clavulanic acid	1	8%	1	8%	10	83%
Lincomycin	2	17%	2	17%	8	67%
Ciproflxacin	6	50%	3	25%	3	25%
Amikacin	1	8%	8	67%	3	25%

The results of this study showed that the effect of the inhibitory garlic on the isolates which is much more than the effect of the antibioticsusedin which that some isolates showed resistance against them such as cefuroxime sodium, cefotaxime, amoxicillin, clavulanic acid, and lincomycinwith the percentages of resistance against these antibiotics are respectively 100%, 92%, 83%, 67%, table (2).

DISCUSSION

Staphylococcus aureus and *Pseudomonas aeruginosa* are considered to be very common acquired infections in hospitals and communities, and the rate of infection is increasing, the potency and efficiency of some antibiotics to treat such infections became very limited due to the developed mechanisms of resistance of these bacteria against such antibiotics (Obritsch *et al.,* 2005; Maltezou and Giamarellou, 2006). The antimicrobial activity of garlic has been attributed to the presence of allicin (Ruddock *et al.,* 2005), in which it has biological properties and great effects against the microbes (Yutani *et al.,* 2001).Such inhibitory effect of the garlic is due to presence of allins compound which is alklcystinesulfoxidea compound in which such compound converts into another compound named allicine which is known as allylsulfide which plays a vital role of bacterial inhibition (Avato *et al.,* 2000). The mechanism of action of the allicine is to combined with the amino acid cysteine which contains thiol group that specifically act to breakdown the disuifide linkage that are available in the proteins of the bacterial cells, thus it strongly hinders the multiplication of bacteria.

Thiol group interacts with alcohol dehydrogenase, thioredoxin, and RNA polymerase that have an essential effect in the active metabolism of the amino acid cysteine proteinase that are necessary in bacterial pathogenicity (Ayaz and Alpooy, 2007), also garlic has a strong effect to prevent the production of bacterial toxins (Sivam, 2001), therefore garlic is considered to be a very potent inhibitory agent against growth of many Gram positive and Gram negative bacteria such as *Staphylococcus aureus* (Cutlerand Wilson, 2004), *Pseudomonas aeruginosa* (Abouelfetouh and Moussa, 2012), *Campylobacter jejuni* (Lu *et al.,* 2011), *Escherichia coli* (Durairaj *et al.,* 2009), Salmonella (Belguith*et al.,* 2010) and *Helicobacter pylori* (Sivam, 2001),sothe garlic is a broad spectrum antibiotic to treat many microbial infections due to the effect of allicin which inhibits the growth of these microbes (Ayaz and Alpooy, 2007).

In this study,garlic was observed to have an antimicrobial effect against all the bacterial isolates used, similar as the broad spectrum antibiotics,garlic exhibited broad spectrum activity against Gram positive and Gram negative bacteria (Ross *et al.,* 2001). Bothgarlic and ciprofloxacin were effective antibiotics against the growth of isolates in the study in which they have similar effect against*Staphylococcus aureus* and *Pseudomonas aeruginosa*, so allicin is the essential effective substance that acts to have a partial inhibition of DNAand protein synthesis, whereas it totally inhibits the synthesis of RNA of bacterial cell, suggesting that RNA is the primary target of allicin (Eja *et al.,* 2007). Ciprofloxacinacts to inhibit the DNA gyrase enzyme of the bacterial cell, there is also a similarity regarding the mechanism of action of the garlic and the antibiotics that belong to penicillins and cephalosporins groups that inhibit the formation of the cell wall of bacteriaby the effect of the transpeptidations that are required for the linkage of polysaccharide chains forms the peptidoglycan layer of bacterial cell wall (Brooks*et al.,* 2010). This study revealed that high sensitivities of garlic for the bacterial isolates more than antimicrobial agents used, in which some isolates showed high resistance to antimicrobial agents such as cefuroxime sodium, cefotaxime, amoxicillin/clavulanic acid and lincomycin,so, the garlic has a potent effect against the microbes that have a resistance against the antibiotics (Durairaj *et al.,* 2009; Iwalokun *et al.,* 2004 ; Tsao and Yin, 2001),whereas *Staphylococcus aureus* and *Pseudomonas aeruginosa* areconsidered to have a wide resistance against several antibiotics (Okesola and Oni, 2009 ; Javeed *et al.,* 2011 ; Ash*et al.,* 2002),garlic is an antibiotic even against the microbes that have a multi-resistance effect against antibiotics (*Fani et al.,* 2007), in which other studies showedthat high percentage for the resistance of antibiotics against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Radha *et al.*, 2012 ; Masoud *et al.,* 2011).

Conclusion

The inhibitory effect for the aqueous garlic extract is more effective than the inhibitory effect of the antimicrobial agents used in the study. The effect of the garlic is quite similar to the inhibitory mechanism of some antimicrobial agents such as ciprofloxacin which mainly acts to inhibit the synthesis of RNA and DNA gyrase enzyme in the bacterial cell, furthermore, the garlic acts to inhibit the synthesis of peptidoglycan which forms the cell wall of the bacteria.The aqueous garlic extract is the most selected and acceptable to inhibit the growth of Gram positive and Gram negative bacteria.The absence of resistance to garlic enhances its ability to effectively act against resistant and multi-drug resistant bacterial strains such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

REFERENCES

Abouelfetouh, A.Y. and Moussa, N.K. 2012. Enhancement of antimicrobial activity of four classes of antibiotics combined with garlic. *Asian journal of plant science*,11(3), 184-152.

Ash, R.J., Mauck, B. Morgan, M. 2002. Antibiotic resistance of Gram-negative bacteria in rivers, United States. Emerging Infectious Diseases, 8(7), 713-716.

Avato, P., Tursi, F., Vitali, C., Miccolis, V. Candido, V. 2000. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. Phytomedicine, 7(3), 239-243.

Ayaz, E. Alpooy, H.C. 2007. Garlic (*Allium sativum*) and traditional medicine. TurkiyeParazitolDerg, 31(2), 145-149.

Bakri, I.M. Douglas, C.W. 2005. Inhibitory effect of garlic extract on oral bacteria. Arch. Oral Biol, 50, 645-651.

Belguith, H., Kthiri, F., Chati, A., Abu Sofah, A., Ben Hamida, J. Landoulsi A. 2010. Study of the effect of aqueous garlic extract (*Allium sativum*) on some Salmonella serovars isolates. Emir. J. Food Agric, 22(3), 189-206.

Bosso, J.A. 2005. The antimicrobial armamentarium: evaluating current and future treatment options. Pharmacotherapy, 25, 55S-62S.

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A. Mietzner, T.A. 2010. Ch. 28: Antimicrobial chemotherapy. InJawetz, Melnick and Adelberg 's Medical microbiology (pp. 339-371). New York: McGraw-Hill Companies.

Brown, J.C., Huang, G., Haley-Zitlin, V. Jiang, X. 2009. Antibacterial effects of grape extracts on *Helicobacter pylori*. Applied and Environmental Microbiology, 75(3), 848-852.

Cai, Y., Wang, R., Pei, F. Liang, B.B. 2007. Antibacterial activity of allicin alone and in combination with β -lactams against *Staphylococcus* spp. and *Pseudomonas aeruginosa.* J. Antibiotic, 60(5), 335-338.

Clinical and laboratory standards institute, CLSI. 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition, 32(1), 8-13.

Cutler, R.R. Wilson, P. 2004. Antibacterial activity of a new stable aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. British J. Biomedical Science, 61(2), 1-4.

Durairaj, S., Srinivasan, S. Lakshmanaperumalsamy, P. 2009. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Electronic Journal of Biology, 5(1), 5-10.

Eja, M.E., Asikong, B.E., Abriba, C., Arikpo, G.E., Anwan, E.E. Enyi-Idoh, K.H. 2007. A comparative assessment of the antimicrobial effects of garlic (*Allium stavium*) and antibiotics on diarrheagenic organisms. Southeast Asian J. Trop. Med. Public Health, 38 (2), 343-348.

*Fani***,** M.M*., Kohanteb*, J., *Meshki***,** K., *Ghotbabadi,* E.S., *Sobhnamayan*, F. *Dayaghi,* M. 2007. Inhibitory effect of aqueous garlic (*Allium sativum*) extract on multi-drug-resistant *Streptococcus mutans* species: an *in vitro* study. Journal of Isfahan dental school, 6(4), 123-197.

Hauser, A.R. Sriram, P. 2005. Severe *Pseudomonas aeruginosa*infections. Tackling the conundrum of drug resistance.Postgrad. Med, 117, 41-48.

Iwalokun, B.A., Ogunledun, A., Ogbolu, D.O., Bamiro, S.B. 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), 327-333.

Javeed, I., Hafeez, R. Anwar, M.S. 2011. Antibiotic susceptibility pattern of bacterial isolates from patients admitted to a tertiary care hospital in Lahore. Biomedica, 27, 19-23.

Lemar, K.M., Passa, O., Aon, M.A., Cortassa, S., Müller, C.T., Plummer, S., O'Rourke, B. Lloyd, D. 2005. Allyl alcohol and garlic (*Allium sativum*) extract produce oxidative stress in *Candida albicans*. Microbiology, 151, 3257-3265.

Lu, X., Rasco, B.A., Jabal, J.M.F., Aston, D.E., Lin, M. Konkel, M.E. 2011. Investigating antibacterial effects of garlic (*Allium sativum*) concentrate and garlic-derived organosulfur compounds on *Campylobacter jejuni* by using fourier transform infrared spectroscopy, Raman Spectroscopy, and Electron Microscopy. Applied and Environmental Microbiology, 77(15), 5257–5269.

Maltezou, H.C. Giamarellou, H. 2006. Community-acquired methicillin-resistant *Staphylococcus aureus*infections. Int. J. Antimicrob. Agents, 27, 87-96.

Masoud, E.A., Mahdy, M.E. Esmat, A.M. 2011. Bacterial prevalence and resistance to antimicrobial agents in southwest, Saudi Arabia. Egypt. Acad. J. Biolog. Science, 3(1), 105-111.

Obritsch, M.D., Fish, D.N., MacLaren, R. Jung, R. 2005. Nosocomial infections due to multidrug-resistant *Pseudomonasaeruginosa*: epidemiology and treatment options. Pharmacotherapy, 25, 1353–1364.

Okesola, A.O. Oni, A.A. 2009. Antimicrobial resistance among common bacterial pathogens in south western Nigeria. American-Eurasian J. Agric. & Environ. Science, 5(3), 327-330.

Radha, K., Uma, R., Ramanathan, G. Thangapandian, V. 2012. Evaluation of bacterial isolates from clinical samples for multi-drug resistance. J. Microbiol. Biotech. Research, 2(1), 138-146.

Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleightholme, H.V. Maslin, I.J. 2001. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbiology, 67, 475-480.

Ruddock, P.S., Liao, M., Foster, B.C., Lawson, L., Arnason, J.T. Dillon, J.A. 2005. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against *Neisseria gonorrhoeae*, *Staphylococcus aureus*and *Enterococcus faecalis*. Phytother. Research, 19, 327-334.

Shams-Ghahfarokhi, M., Shokoohamiri, M.R., Amirrajab, N., Moghadasi, B., Ghajari, A., Zeini, F., Sadeghi, G. Razzaghi-Abyaneh, M. 2006. *In vitro*, antifungal activities of *Allium cepa*, *Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia, 77(4), 321-323.

Sivam, G.P. 2001. Protection against *Helicobacter pylori* and other bacterial infections by garlic. J. Nutrition,131(3), 1106-1108.

Tsao, S-M, and Yin, M.C. 2001. *In vitro,* activity of garlic oil and four diallylsulphides against antibiotic resistant *Pseudomonas aeruginosa* and *Klebsiellapneumoniae*. Journal of antimicrobial chemotherapy, 47, 665-670.

Yutani, M., Taniguchi, H., Borjihan, H., Ogita, A., Fujita, K. Tanaka, T. 2011. Alliinase from ensiferadhaerens and its use for generation of fungicidal activity. AMB Express, 1(2), 1-8.

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