In-vitro study of inhibitory effect of garlic extract on *Aeromonas sobria*

Amiri Farahani, M.[1*], Peyghan. R.[1], Motamedi, H.[2]

[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*

[2]*Biology Department, Sciences Faculty of Shahid Chamran University of Ahvaz, Ahvaz, Iran.*

Key words:
Garlic extracts, *Aeromonas sobria*, Common carp, Bacteria.

Correspondence
Amiri Farahani, M.
DVM student, Faculty of Veterinary, Shahid Chamran University, Ahvaz, Iran.
Tel: +98(611) 3364378
Fax: +98(611) 3360807
Email: m_amirifarahani@yahoo.com

Received: 15 May 2012
Accepted: 18 July 2012

Abstract:

BACKGROUND: Garlic (*Allium sativum*) is known for its anti-bacterial properties, but information on its effects against bacteria species that are important in fish diseases is scarce. **OBJECTIVES:** The aim of this research was to use garlic as a natural product to improve the aquatic animal health status so as to compensate the demand for environment-friendly products for sustainable aquaculture. **METHODS:** For this purpose thee methods of extraction including: row garlic extract, water, ethanol and methanol extract were used for in-vitro toxicity tests on Aeromonas sobria by disk diffusion and tube test. **RESULTS:** According to results in 200 and 400 mg/mL concentrations of ethanol extract of garlic, the inhibition zone of bacterial growth was 7 and 10 mm respectively. There was no inhibition zone for all concentrations of methanol extract of garlic. In water garlic extract the inhibition zone for concentrations of 100, 200 and 400 mg/mL were 8, 10 and 14 mm respectively. For 100% and 50% raw garlic the inhibition zone was 27 and 8 mm respectively. MIC for Aeromonas sobria in ethanol extract, water extract and raw garlic were estimated as 200, 100 mg/mL and 10% respectively. MBC for these extracts was also estimated as 300, 100 mg/mL and 25% respectively. **CONCLUSIONS:** Our data indicate that raw and water extract of garlic have the highest antibacterial effect. Ethanol extract had a lower effect and methanol showed no bacteriostatic effect. Therefore, garlic extract can inhibit the growth of Aeromonas sobria, an important fish pathogen, and may have therapeutic value, particularly for carp.

Introduction

Garlic (*Allium sativum*) is known as a potent medicine with broad therapeutic properties ranging from antibacterial (Martin and Ernst, 2003; Ross et al., 2001; Ross and Yin, 2001) to antivirus (Shoji et al., 1993; Weber et al., 1992), anticancer (Harris et al., 2001), antifungal(Mousavi et al., 2008) and anti-protozoa (Peyghan et al., 2008; Harandi et al., 2006). The fresh garlic bulb has sulfur-containing compounds such as allicin, which has many medicinal properties. Quantitative tests revealed that there were 17.8 n moles sulfhydryl (SH)/mL of 40 KD garlic proteinlocalized in the parenchyma sheath cells and the cortical cells of garlic bulbs (Wen et al., 1995). Garlic has long been known to have anti-bacterial properties, but there is little data on its effects against bacteria species that are important in fish diseases. With the rise in bacterial resistance to antibiotics there is considerable interest in the development of other antimicrobials for the control of infection. The main antimicrobial constituent of garlic has been identified as the sulphur compounds and allicin. Allicin is the major thiosulphinate compound found in garlic homogenate (Banerjee et al., 2003). It is the

one of the active principles of freshly crushed garlic homogenates that was found to exhibit antibacterial and antiparasitic activity by means of chemical reaction with thiol groups of various enzymes (Martin and Ernst, 2003). Inhibito-lethal activity against Giardia intestinalis was noted with crude extract of garlic at 25 µg mL^{-1} and lethal dosage was established as 50 µg mL^{-1}(Harris et al., 2001).

With the increase in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. More recently, garlic has been proven to be effective against the growth of gram-positive, gram-negative and acid -fast bacteria (Harris et al., 2001). The fresh garlic has sulfur containing compounds such as allicin, which is an odorless material (Ankri and Mirelman, 1999; Peyghan et al., 2008). Allicin itself is very unstable and decomposes rapidly (Brodnitz et al., 1971). Typical garlic contains about 1% allicin. Other key materials are allyl methyl thiosulphonate, 1-propenyl allylthiosulohanate and L-glutamyl-S-alkyl-L-cysteine (Fenwich and Hanley, 1985). Indeed, garlic extract has been shown to have a wide spectrum of antibacterial activity including, effects on *Escherichia* and *Salmonella* (Johnson and Vaughn, 1969), Staphylococcus (Cavallito and Bailey, 1994), *Klebsiella* (Jezowa et al., 1966), Proteus, *Clostridium*, *Mycobacterium* (Delaha and Garagusi, 1985) and *Helicobacter species* (Jonkers et al., 1999). In addition, certain oral *Streptococcus mutans* have been shown to be sensitive to garlic extract and a mouth wash containing garlic extract was more effective at reducing the total salivary bacterial count and the mutans streptococcal count (Fani et al., 2007). The aim of this study was, therefore, to investigate the antimicrobial effect of garlic extract on an important bacteria which usually causes disease in freshwater fishes. In this research garlic was used as a natural product for improving the aquatic animal health status to compensate the demand for environment-friendly products for sustainable aquaculture.

Material and Methods

Bacterial preparation: The tests were conducted using a clinical isolate of *Aeromonas sobria*. The *Aeromonas sobria* was isolated and had been identified previously from a diseased common carp by means of biochemical and serological tests.

Garlic preparation: For this purpose raw garlic extract, water, ethanol and methanol extract were used for in-vitro toxicity tests. Raw garlic homogenate was prepared by crushing the garlic in a pestle and mortar. For water extract, ethanol extract and methanol extracts, dry garlic bulbs were crushed and mixed with water, ethanol and methanol respectively by clean mixer. The preparations were left to dry at room temperature and the sediments were collected separately.

Diffusion test: Organisms were spread on Mueller-Hinton agar for the tests and were coded and tested blindly by disk diffusion test method. The prepared extracts were used in various concentrations of garlic and antibiotics. For this purpose 4 types of garlic extract were used (crud extract, water extract, methanol and ethanol extract) in three replicates each. For crud extract 100% and 50% garlic extract were used and for water extract, methanol and ethanol extract 3 concentrations (100, 200 and 400 mg/mL) were used for each experiment. In this method, for disc inhibition with extracts, 50 µL of extract was added to each disc paper. For the controls the discs were imbibed by water, methanol and ethanol respectively. Also, crude garlic extracts were serially diluted in Mueller-Hinton agar in tubes and each was inoculated with 0.5 degree of McFarland bacterial cell suspension. For determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), 100 µL of each concentration of garlic extract was added to 1 mL of Muller-Hinton broth containing *Aeromonas sobria* in concentration equal to 0.5 scales of McFarland. Tubes and plates were incubated at 37 °C for 24 hours and the highest dilution where there was no growth and bactericidal effect were recorded as the MIC and MBC respectively (Ankri and Mirelman, 1999). As a negative control, distilled water, methanol and ethanol were used for comparison (the blank disk). As the positive control, a disk of Trimethprim sulfamethoxazole, Amikacin, Oxytetracyclin, Streptomycin and Amoxicillin were used.

Results

According to results in the 200 and 400 mg/mL

concentrations of ethanol extract of garlic, the inhibition zone of bacterial growth was 7 and 10 mm respectively. There was no inhibition zone for all concentrations of methanol extract of garlic. In water garlic extract the inhibition zone (Figure 1) for concentrations of 100, 200 and 400 mg/mL were 8, 10 and 14 mm respectively. For 100% and 10% raw garlic the inhibition zone was 27 and 8 mm respectively.

MIC for *Aeromonas sobria* in ethanol extract, water extract and raw garlic was estimated as 255, 100, mg/mL and 10% respectively (Table 1). MBC for these extracts were also estimated as 300, 100 mg/mL and 25% respectively. Our data indicate that raw and water extract of garlic had the highest antibacterial effect. The ethanol extract had a lower effect and methanol showed no bacteriostatic effect.

As negative control, distilled water, methanol and ethanol (blank discs) showed no growth inhibition zone. As the positive control, a disk of Trimethprim sulfamethoxazole, Amikacin, Oxytetracyclin, Streptomycin and Amoxicillin had diameters of 24mm, 24mm, 12mm, 20mm and 9mm respectively.

Discussion

The results showed that crude extract of garlic has the best and most powerful anti-bacterial effect on *Aeromonas sobria* where the diameter of outgrowth of the halo was 27 mm. The anti-bacterial effect of garlic is assignable to the allicin. Garlic has played an important medicinal role for centuries. Garlic herb is another kind of vegetable which has an effective anti-microorganism nature that can be used for the treatment of many diseases with its curative nature. Allicin has a very changable structure and dis-integrates rapidly, so it can be stated that fresh garlic has a greater anti-bacterial effect than other types of garlic. It has been reported that garlic extract showed in-vitro activity against viruses such as influenza A and B (Fenwich and Hanley, 1985), cytomegalovirus ((Meng et al., 1993), rhinovirus, HIV, herpes simplex1 (Tsai et al., 1985) and 2 (Weber et al., 1992), viral pneumonia and rotaviruses (Harris et al., 2001). Mousavi et al. (2008) showed that garlic gel was used in an investigation against ringworm disease and a 75-77% treatment success was seen. Peyghan et al. (2008) showed that garlic extract had a marked effect on the protozoan parasite, Neoparamoebapema-quidensis.

Figure 1. Inhibitory zone of water garlic extract.

Table 1. Inhibitory zoon, MIC and MBC concentration in different garlic treatments. (*) This concentration is MIC or MBC respectively.

Extract	Inhibition zoon	MIC	MBC
100% crud	27mm	-	-
50% crud	8mm	-	-
25% crud	-	-	*
10% crud	0	*	-
400 mg/mL ethanol extract	10mm	-	-
300 mg/mL ethanol extract	-	-	*
200 mg/mL ethanol extract	7mm	*	-
100 mg/mL ethanol extract	0	-	-
400 mg/mL aqueous extract	14mm	-	-
300 mg/mL aqueous extract	-	-	-
200 mg/mL aqueous extract	10mm	-	-
100 mg/mL aqueous extract	8mm	*	*
Amikacin	24mm	-	-
Oxytetracyclin	12mm	-	-
Streptomycin	20mm	-	-
Amoxicillin	9mm	-	-
Trimethprimsulfamethoxazol	24mm	-	-

Trimethoprim sulfamethoxazole was used in this survey and is categorized as a sulfonamide drug. Trimethprim sulfamethoxazole is a complex product of trimethoprim sulfate and trimethoprime. The spectrum of sulfonamide compound with tri-methoprim is vast and reduces the bacterial resistance risk. However, the antibacterial effect of fresh garlic extract was shown to be more than Trimethprim sulfamethoxazole.

MIC for *Aeromonas sobria* in ethanol extract, water extract and raw garlic was estimated as 255, 100, mg/mL and 10% respectively. In another study it

was shown that MICs and MBCs of aqueous allicin extract were 35-95 mg/l and 75-315 mg/l. Time/dose kill curves produced a 2-3 log reduction in cfu/mL within 3h and no detectable growth at 8 and 24h (Cutler et al., 2009). Mousavi et al. (2008) explained that MIC of the aqueous and methanol extract of garlic on *Trichophyton mentagrophytes* and *Microsporum canis* was the same; however, the effect of methanol on *Microsporum gypseum* was more than that of the aqueous extract. Vaseeharan et al. (2011) showed the in-vitro and in-vivo inhibitory effects of *Allium sativum* extract evaluated in a multidrug-resistant survey by Vibroharveyi. In that study freshly squeezed garlic extract showed greater inhibitory activity against V. harveyi than freeze-dried garlic extract and methanolic garlic extract.

In this research it is proved that the use garlic extract can prevent bacterial growth. This survey showed the clear effect of garlic extract on the *Aeromonas sobria* agent of bacterial septicemia disease in fresh water fishes. Although methanol extract does not have an anti-bacterial effect, water and ethanol extracts have a fairly good anti-bacterial effect where water extract is superior to ethanol. Therefore, according to our results, garlic extract can inhibit the growth of *Aeromonas sobria*, an important fish pathogen and may have therapeuticvalue, hence it can be suggested for control of this disease, particularly for carp. There are many studies on garlic properties in Iran and other countries; however, our study is the first study that concentrates on fish pathogens. In our study garlic extract had an obvious antibacterial effect and can be an an effective treatment using natural sources. It would also be less expensive than some other synthetic drugs. Moreover, this kind of product may be safe for fish and have less impact on the environment.

Acknowledgment

This research was supported (Grant, 1390) by the Research Deputy of Shahid Chamran University, Ahvaz, Iran and all efforts are appreciated.

References

1. Ankri, S., Mirelman, D. (1999) Antimicrobial properties of allicin from garlic. Microb. Infect. 1: 125-129.
2. Banerjee, S., Mukherjee, P.K., Maulik, S. (2003) Garlic as an antioxidant: the good, the bad and the ugly. Phytother. Res. 17: 97-106.
3. Brodnitz, M.H., Pascale, J.V., Van Derslice, L. (1971) Flavor components of garlic extract. J. Agric. Food Chem. 19: 273-275.
4. Cavallito, C.J., Bailey, J.H. (1944) Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66: 1950-1951.
5. Cutler, R.R., Odent, M., Hajjahmad, H., Maharijan, S., Bennett, N.J., Josling, P.D., et al. (2009) In vitro activity of an aqueous allicin extract and a novel allicin topical gel formulation against Lancefield group B streptococci. J. Antimicrob. Chemother. 63: 51.
6. Delaha, E.C., Garagusi, V.F. (1985) Inhibition of mycobacteria by garlic extract (*Allium sativum*). Antimicrob. Agents Chemother. 27:485.
7. Fani, M., Kohanteb, J., Dayaghi, M. (2007) Inhibitory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans*. J. Indian Soc. Pedodont. Prev. Dent. 25: 164.
8. Fenwich, G., Hanley, A. (1985) The genus Allium. CRC Rev Food Sci Nuri. 22: 199-377.
9. Harandi, M.S., Dalimiasl, A., Ghaffarifar, F. (2006) In ivitro and in vivo effects of garlic(*Allium sativum*) extract on Giardia lamblia and Giarda muris. Hakim Research Journal. 9: 53-64.
10. Harris, J., Cottrell, S., Plummer, S., Lioyd, D. (2001) Antimicrobial properties of *Allium sativum* (garlic). Appl. Microbiol. Biotechnol. 57: 282-286.
11. Harris, J., Plummer, S., Lloyd, D. (2001) Antigiardial drugs. Appl. Microbiol. Biotechnol. 57: 614-619.
12. Jezowa, L., Rafinski, T., Wrocinski, T. (1966) Investigations on the anitibiotic activity of *Allium sativum* L., Herba Pol. 12: 3-13.
13. Johnson, M.G., Vaughn, R.H. (1969) Death of *Salmonella typhimurium* and *Escherichia coli* in the presence of freshly reconstituted dehydrated garlic and onion. Appl. Environ. Microbiol. 17: 903.
14. Jonkers, D., Van Den Broek, E., Van Dooren, I., Thijs, C., Dorant, E., Hageman, G., et al. (1999) Antibacterial effect of garlic and omeprazole on

Helicobacter pylori. J. Antimicrob. Chemother. 43: 837.

15. Martin, K.W., Ernst, E. (2003) Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. J. Antimicrob. Chemother. 51: 241.
16. Meng, Y., Lu, D., Guo, N., Zhang, L., Zhou, G. (1993) Anti-HCMV effect of garlic components. Virol Sin. 8: 147-150.
17. Mousavi, S.A.A., Yaghmai, B., Mehrabian, M. (2008) The study of the effects of aqueous and methanol extracts of garlic against *Trichophyton Mentagrophytes*, *Microsporum canis* & *Microsporum Gypseum*. J. Rafsanjan Uni. Med. Sci. 8: 3-10.
18. Peyghan, R., Powell, M., Zadkarami, M.R. (2008) In vitro effect of garlic extract and metronidazole against Neoparamoeba pemaquidensis, page 1987 and isolated amoebae from Atlantic salmon. Pak. J. Biol. Sci. PJBS. 11: 41.
19. Ross, Z., O'Gara, E.A., Hill, D.J., Sleightholme, H.V., Maslin, D.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. Microbiol. 67: 475.
20. Shoji, S., Furuishi, K., Yanase, R., Miyazaka, T., Kino, M. (1993) Allyl compounds selectively killed human immunodeficiency virus (type 1)-infected cells. Biochem. Biophys. Res. Commun. 194: 610-621.
21. Tsai, Y., Cole, L.L., Davis, L.E., Lockwood, S.J., Simmons, V., Wild, G.C., et al. (1985)Anti viral properties of garlic : in vitro effects of influenza B, herpes simplex virus, and coxsackie viruses. Planta Med. 51: 460-461.
22. Tsao, S., Yin, M. (2001) In vitro activity of garlic oil and four diallyl sulphides against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. J. Antimicrob. Chemother. 47: 665.
23. Vaseeharan, B., Prasad, G.S., Ramasamy, P., Brennan, G. (2011) Antibacterial activity of *Allium sativum* against multidrug-resistant Vibrioharveyi isolated from black gill-diseased Fenneropenaeus indicus. Aquacult. Int. p. 1-9.
24. Weber, N.D., Andersen, D.O., North, J.A., Murray, B.K., Lawson, L.D., Hughes, B.G. (1992) In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Medica. 58: 417-417.
25. Wen, G., Mato, A., Wisniewski, H.M., Malik, M.N., Jenkins, E.C., Sheikh, A.M., et al. (1995) Light and electron microscopic immunocytochemical localization of two major proteins in garlic bulb. J. Cell. Biochem. 58: 481-489.

مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۴، ۲۱۷-۲۱۳

مطالعه آزمایشگاهی اثر مهارکنندگی عصاره سیر بر روی آئروموناس سوبریا

مینا امیری فراهانی[1*] رحیم پیغان[1] حسین معتمدی[2]

۱) گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران.
۲) گروه میکروبیولوژی، دانشکده علوم دانشگاه شهید چمران اهواز، اهواز، ایران.

(دریافت مقاله: ۲۶ اردیبهشت ماه ۱۳۹۱ ، پذیرش نهایی: ۲۸ تیر ماه ۱۳۹۱)

چکیده

زمینه مطالعه: خاصیت ضدباکتریایی گیاه سیر از زمان‌های گذشته شناخته شده است اما گزارشی در مورد خواص ضدباکتریایی سیر بر باکتری‌های ایجادکننده بیماری‌های ماهی وجود ندارد. **هدف:** هدف از این مطالعه استفاده سیر به عنوان ماده طبیعی برای پیشرفت وضعیت سلامت آبزیان می باشد. **روش کار:** برای این منظور عصاره خام سیر، آبی، اتانولی و متانولی برای مطالعه سمیت در شرایط آزمایشگاهی روی باکتری آئروموناس سوبریا به وسیله دیسک دفیوژن و لوله آزمایش استفاده شد. **نتایج:** با توجه به نتایج بدست آمده در غلظت ۲۰۰mg/mL و ۴۰۰ عصاره اتانولی سیر، به ترتیب هاله عدم رشد ۷mm و ۱۰ گزارش شد. هیچ هاله عدم رشدی برای تمام غلظت‌های متانولی عصاره سیر یافت نشد. در عصاره آبی سیر هاله عدم رشد برای غلظت ۲۰۰mg/mL، ۱۰۰ و ۴۰۰ به ترتیب ۸mm، ۱۰ و ۱۴ گزارش شد. برای عصاره خام سیر در غلظت ۵۰٪ و ۱۰۰٪ هاله عدم رشد ۸mm و ۲۷ بود. MIC آئروموناس سوبریا برای عصاره اتانولی، عصاره آبی به ترتیب ۲۰۰mg/mL و ۴۰۰ و برای عصاره خام سیر ۱۰٪ برآورد شد. MBC برای این عصاره به ترتیب ۳۰۰mg/mL و ۱۰۰ و برای عصاره خام سیر ۲۵٪ تخمین زده شد. **نتیجه‌گیری نهایی:** اطلاعات ما نشان داد که عصاره خام و آبی سیر دارای بیشترین اثر ضد باکتریایی می‌باشد. عصاره اتانولی دارای کمترین اثر آنتی باکتریال و عصاره متانولی فاقد اثر آنتی باکتریال می‌باشد. بنابراین عصاره سیر می‌تواند رشد آئروموناس سوبریا را که یک پاتوژن مهم ماهی است مهار کرده و ارزش درمانی داشته باشد.

واژه‌های کلیدی: عصاره سیر، آئروموناس سوبریا، باکتری، ماهی کپور.

*)نویسنده مسؤول: تلفن: ۳۳۶۴۳۷۸ (۶۱۱) ۹۸+ نمابر: ۳۳۶۰۸۰۷ (۶۱۱) ۹۸+ *Email: m_amirifarahani@yahoo.com*