http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (8): 1577-1579, 2006
ISSN 1028-8880
© 2006 Asian Network for Scientific Information

Antibacterial Effect of Garlic (*Allium sativum* L.) on *Staphylococcus aureus*

[1]M. Shokrzadeh and [2]A.G. Ebadi
[1]Department of Toxicology, Faculty of Pharmacy,
Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Biology, Islamic Azad University,
Sari branch, 48164-194, Iran

Abstract: Garlic (*Allium sativum* L.) has an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which grows in most of Europe and in northern Iran. Iranian garlic is used in traditional medicine for infectious diseases, flu and as an anti-febrile. The present study tested the aqueous extract of garlic in vitro for its antibacterial activity. The extract showed concentration-dependent antibacterial activity against *Staphylococcus aureus* 8327. This activity was heat resistant, but the activity of freeze-dried extract gradually diminished during a 90 days period. The traditional use of Iranian garlic for infectious diseases and for controlling fever appears to be justified.

Key words: Garlic, antibacterial activity, *Staphylococcus aureus*

INTRODUCTION

Garlic has had an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects are ascribed to specific oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic (Block, 1985). During crushing or cutting of the clove, the odorless amino acid alliin, present in the garlic clove, is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to yield allicin and other thiosulfinates that are the source of the characteristic odor of garlic. Thiosulfinates and other secondary metabolites of garlic, including 7-glutamyl peptides, scordinins, steroids, terpenoids, flavonoids and other phenols, may be responsible for the range of therapeutic effects reported for garlic. Reuter *et al.* (1996) recently reviewed the therapeutic effects of garlic, namely, effects on the cardiovascular system, antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and hormone-like effects. This study will focus on recent research on protective effects of garlic against Helicobacter pylori and other bacterial infections.

There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and alcoholic extracts, lyophilized powders, steam distilled oil and other commercial preparations of garlic. Fenwick and Hanley (1985) undertook a thorough review of the antibacterial effects of garlic and other allium vegetables up to mid-1984; more recently, the antibacterial effects of garlic have been studied by Reuter *et al.* (1996). Garlic has been reported to inhibit *Aerobacter*, *Aeromonas*, *Bacillus*, *Citrella*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Mycobacterium*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Vibrio*. Noteworthy among the reported findings are the following: 1) Garlic exhibits a broad antibiotic spectrum against gram-positive and gram-negative bacteria (Kabelik and Hejtmankova-Uhrova, 1968). 2) Enterotoxic coli strains and other pathogenic intestinal bacteria, which are responsible for diarrhea in humans and animals, are more easily inhibited by garlic than the normal intestinal flora (Caldwell and Danzer 1988; Kumar and Sharma, 1982; Rees *et al.*, 1993). 3) Garlic is active even against organisms that have become resistant to antibiotics (Jezowa *et al.*, 1966). 4) The combination of garlic extracts with antibiotics leads to partial or total synergism (Didry *et al.*, 1992). 5) A garlic oil preparation showed good antituberculosis activity in guinea pigs with a intraperitoneal dose of 0.5 mg kg^{-1} (Jain, 1998). 6) Complete lack of resistance of bacteria to garlic has been found (Dankert *et al.*, 1979; Singh and Shukla, 1984). 7) As a result of the bactericidal activity of garlic, toxin production by the bacteria is also prevented (De witt *et al.*, 1979).

The present study tested an aqueous extract of dried garlic *in vitro* for its antibacterial activity against *Staphylococcus aureus* 8327.

MATERIALS AND METHODS

Plant and extract: The Garlic found in the north of Iran which is different from the garlic grown in Europe. Dried garlic were collected from Mazandaran province,

Corresponding Author: A.G. Ebadi, Department of Biology, Islamic Azad University, Sari branch, sari 48164-194, Iran

in northern Iran in mid August (2004). Cold aqueous extract (pH 5.8) of dried *Allium sativum* (5%, w/v) was used in all the experiments. Dried garlic (15 g) were steeped for 6 h at 4°C in 300 mL distilled water, with constant stirring. The material was centrifuged and the supernatant was filter-sterilized and then freeze-dried.

Anti-bacterial effect of extract: *Staphylococcus aureus* 8327 was obtained from Tehran University of Medical Sciences, Faculty of Health, Iran. Nutrient broth (NB) containing 5 g peptone (Difco), 5 g NaCl and 3 g beef extract in 1 L of distilled water (pH 7.5) was used as a culture medium. Anti-bacterial activity of the extract was determined by agar-well diffusion, disc diffusion based on Jezowa *et al*. (1966), and the minimum inhibitory concentration (MIC) methods (Caldwell and Danzer, 1988). In the agar-well diffusion method, 9 mm diameter wells were prepared on agar containing 0.5 mL of bacteria ($2x10^{11}$ cells mL^{-1}). Freeze-dried extract was diluted 1:20 and different concentrations (1.25 to 10 mg) were added to the wells. In the disc diffusion method, paper discs were soaked in extract solutions and were placed on the bacteria. After 24 h at 37°C, the inhibition zones were measured.

To determine MIC, 5 mL medium was added to six tubes. In the first tube 5 mL extract (1:20 dilution, 50 mg mL^{-1}) was added and after mixing 5 mL was removed and added to the second tube; the dilutions continued for all the tubes. Then, 14 mL medium and 1 mL bacteria suspension were added and the tubes were incubated for 24 and 48 h at 37°C.

Chromatography: Thin layer chromatography was used to identify the active ingredients of the aqueous extract. Chromatography was performed for 15 h using butanol:acetic acid:distilled water (5:1:4) solvent on a Whatman #1 filter paper. Spots were stained with ninhydrin (to detect amino acids and flavenoids), bismuth iodine, 3% ferric chloride (to detect esters of carboxylic acids and anhydrides), and with Fehling's A+B solution (Cellini *et al.*,1966).

RESULTS

To determine the antibacterial effect of garlic, an aqueous extract was prepared. To get the best aqueous extraction, distilled water with three different pH 5.8, 7.0 and 8.5, was used, and about 8.2, 6.8 and 7.0 g lyophilized powder were obtained, respectively, from 15 g dried flowers. The agar-well diffusion method with 1:20 dilution of these different extracts gave inhibition zone diameters of 10, 7 and 6 mm at pH 5.8, 7.0 and 8.5, respectively. Therefore, pH 8.5 was selected for extraction. Table 1 shows antibacterial effects of various concentrations of garlic extract with two different methods. The activity was

Table 1: Stability of antibacterial activity of Garlic extract during 90 days by agar-well plate. Freeze-dried extract was prepared (5% W/V) and 0.2 μm was added to the wells.

Time (days)	Inhibition zone diameter (mm)
0	10.0
15	8.9
30	6.2
45	4.0
60	3.8
75	3.0
90	2.0

Table 2: Antibacterial effect of garlic extract on *Staphylococcus aureus*

Method	Extract (mg)	Inhibition zone diameter (mm)
Disc diffusion	4	8
	1	4
	0.5	0
	0.1	0
Agar-well diffusion	10	10
	5	4
	2.5	0
	1.25	0

Table 3: Thin layer chromatography of aqueous garlic extract on Whatman # 1 paper using butanol:acetic acid:water

Reagents	Rf
Ninhydrin	0.13, 0.18, 0.23, 0.38, 0.48, 0.63 (all purple)
Ferric chloride (3%)	0.24 (white), 0. 61 (brown)
Fehling (A+B)	0.21 (yellow)
Bismuth iodine	No spot

bactericidal, since incubation of the inhibition zone for one week did not show any growth of bacteria.

The inhibitory effect of extract was not due to the pH of the extract, since extract with all three pHs of 5.8, 7.0 and 8.5 had antibacterial activity, and the control pH had no effect. These data indicate that the antiviral activity of the extract is due to the garlic component. The MIC of extract on *Staphylococcus aureus* 8327 after 24 and 48 h was determined to be 6.2 mg mL^{-1}. Lower dilutions had no anti-bacterial effect.

The anti-bacterial activity of the extract was heat resistant. Autoclaving the extract at 110°C for one hour did not eliminate its antibacterial activity and the effect was similar to that of the extract that was filter sterilized. When 200 mL of 1:20 dilution of extract was used in 9 mm diameter wells, in both cases the inhibition zones were 12 mm. The stability assay showed that the antibacterial effect of the freeze-dried extract diminished during 90 days storage at 4°C (Table 1), and the activity of the working solution was diminished after one week at 4°C. These results indicate that the traditional use of the Iranian garlic for infectious diseases and for antifebrile activity may be justified. garlic syrup was thought not only to be good for fever, but also to be a remedy for jaundice, itch and ringworm (Cellini *et al.*,1996; Feldberg *et al.*,1988; Fenwick and Hanley, 1985) Also, the study found that garlic extract has anti-viral activity (unpublished data). Already, several components, such as allicinlin and other thiosulfinates that are the source of the characteristic odor of garlic. Thiosulfinates and other

secondary metabolites of garlic, including 7-glutamyl peptides, scordinins, steroids, terpenoids, flavonoids and other phenols, may be responsible for the range of therapeutic effects reported for garlic, have been isolated and characterized (Garau, 1994; Gould, 1994; Graham, 1998; Hughes and Lawson, 1991). In the chromatography experiment, when filter paper was stained with different reagents (Table 2), several spots with different colors and different Rf were obtained. These spots show that the aqueous extract has amino acids (Table 3). No antibacterial activity was identified when these spots were placed on bacterial culture. It seems that the materials in these spots are not enough for anti-bacterial activity; however, more data are needed to determine the active anti-bacterial components of the extract (Lawson, 1996; Sanders and Sanders, 1992; Sivam *et al*., 1997; Steinmetz and Potter, 1991a,b; Tynecka and Gos, 1975).

REFERENCES

Block, E., 1985. The chemistry of garlic and onions. Sci. Am., 252:114-119.

Caldwell, D.R. and C.J. Danzer, 1988. Effects of allyl sulfides on the growth of predominant gut anaerobes. Curr. Microbiol., 16: 237-241.

Cellini, L., E.D. Campli, M. Masuli and S. Di Bartolomeo and N. Allocati, 1996. Inhibition of Helicobacter pylori by garlic extract (*Allium sativum*). FEMS Immunol. Med. Microbiol., 13: 273-277.

Dankert, J., T.F.J. Tromp, H.D. Vries and H.J. Klasen, 1979.Antimicrobial activity of crude juices of *Allium ascalonicum*, *Allium cepa* and *Allium sativum*. Zentrabl. Bakteriol. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Org., 245: 229-239.

De witt, J.C., S. Notermans, N. Gorin and E.H. Kampelmacher, 1979. Effect of garlic oil or onion oil on toxin production by *Clostridium botulinum* in meat slurry. J. Food Prot., 42: 222-224.

Didry, N., L. Dubreuil and M. Pinkas, 1992. Antimicrobial activity of naphthaquinones and *Allium* extracts combined with antibiotics. Pharm. Acta Helv. 67:148-151.

Feldberg, R.S., S.C. Chang, A.N. Kotik, M. Nadler, Z. Neuwirth, D.C. Sundstrom and N.H. Thompson, 1988. *In vitro* mechanism of inhibition of bacterial growth by allicin. Antimicrob, Agents Chemother., 32: 1763-1768.

Fenwick, G. R. and A.B. Hanley, 1985. The genus *Allium*-Part 3. Medicinal effects, CRC Crit. Rev. Food Sci. Nutr., 1: 1-74.

Garau, J., 1994. β-Lactamases: Current situation and clinical importance. Intensive Care Med., 20: 3: S5-S9.

Gould, I.M.,1994. Risk factors for acquisition of multi-drug-resistant gram-negative bacteria. Eur. J. Clini. Micrbiol. Infect. Dis., pp: S30-S38.

Graham, D.Y., 1998. Antibiotic resistance in Helicobacter pylori: Implications for therapy. Gastroenterology, 115: 1272-1277.

Hughes, B.G. and L.D. Lawson, 1991. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* (elephant garlic), and *Allium cepa* (onion), garlic compounds and commercial garlic supplement products. Phytother. Res., 5: 154-158.

Jain, R.C., 1998. Anti tubercular activity of garlic oil. Indian J. Pathol. Microbiol., 41: 131-131.

Jezowa, L., T. Rafinski and T. Wrocinski, 1966. Investigations on the antibiotic activity of *Allium sativum* L., Herba. Pol., 12: 3-13.

Kabelik, J. and N. Hejtmankova-Uhrova, 1968. The antifungal and antibacterial effects of certain drugs and other substances. Vet. Med. (Pragu), 13: 295-303.

Kumar, A. and V.D. Sharma, 1982. Inhibitory effect of garlic (*Allium sativum* Linn.) on enterotoxigenic *Escherichia coli*, Ind. J. Med. Res., 76: 66-70.

Lawson, D.L., 1996. The Composition and Chemistry of Garlic Cloves and Processed Garlic. In: Garlic: The Science and Therapeuti Applications of *Allium sativum* L. and Related Species, 2nd Edn., Koch, H. P. and D.L. Lawson (Eds.), pp: 37-107.

Rees, L.P., S.F. Minney, N.T. Plummer, J.H. Slater and D.A. Skyrme, 1993. A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World J. Micrbiol. Biotechnol., 9: 303-307.

Reuter, H.D., H.P. Koch and D.L. Lawson, 1996. Therapeutic Effects and Applications of Garlic and its Preparations. In: Garlic: The Science and Therapeutic Applications of *Allium sativum* L. and Related Species, 2nd Edn., Koch, H.P. and D.L. Lawson (Eds.), pp:1 35-212.

Sanders, C.C. and W.E. Sanders, 1992. β-lactam resistance in gram-negative bacteria: Global trends and clinical impact. Clin. Infect. Dis., 15: 824-839

Singh, K.V. and N.P. Shukla, 1984. Activity on multiple resistant bacteria of garlic (*Allium sativum*) extract. Fitoterapia, 55: 313-315.

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., 27: 118-121.

Steinmetz, K.A. and J.D. Potter, 1991a. Vegetables, fruit, and cancer. I. Epidemiol. Cancer Causes Control, 2: 325-357.

Steinmetz, K.A. and J.D. Potter, 1991b. Vegetables, fruit, and cancer. II. Mechanisms, Cancer Causes Control, 2: 4527-4542.

Tynecka, Z. and Z. Gos, 1975. The fungistatic activity of garlic (*Allium sativum*) *in vitro*. Ann. Univ. Mariae Curie-Sklodowska Sect. D Med., 30: 5-13.