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The Inhibitory Action of Aqueous Garlic Extract on the Growth of Certain Pathogenic Bacteria

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This work reports the antibacterial effect of aqueous garlic (*Allium sativum*) extract on certain pathogenic Gram positive bacteria, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus pneumonia* and *Streptococcus faecalis*, and Gram negative bacteria *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Acinetobacter haemolyticus* and determined the optimal conditions for storage of garlic extract. The results have revealed that, a concentration of 750 to 1000 µg/ml of the aqueous garlic extract has high antibacterial effect. Storage for 6 h at 30-50 °C, was found to have optimal efficacy for inhibiting the growth of pathogenic bacteria, while a storage temperature of 70-100°C led to loss of the efficacy of aqueous garlic extract.

Key words: Aqueous garlic extract, antibacterial effect, pathogenic bacteria.

INTRODUCTION

Garlic *(Allium sativum)* belongs to the family Liliaceae. This genus contains other kinds such as *A. cepa* and *A. porrum*. The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant [1]. Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium [2]. It contains a higher concentration of sulfur compounds than any other *Allium* species. The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude and aged garlic extract [3]. These compounds are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds, allicin, does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes the amino acid, alliin to allicin [4]. Allicin is further metabolized to vinyldithiines. This breakdown occurs within hours at room temperature and within minutes during cooking [5].

Garlic has a long folkloric history as a treatment for colds, coughs, asthma and is commonly stated to strengthen the immune system [6]. The potent bulb may also be effective against viruses ranging from the common cold to herpes [7,8]. Numerous studies have indicated that garlic extract exhibits broad-spectrum antimicrobial activity against many genera of bacteria and fungi [9,10] with no resistance reported [11]. There are no significant changes on its effects if the garlic has been boiled for five minutes before testing [12]. Garlic is often combined with the herb mullein (*Verbascum* species) in oil products designed to reduce the pain of middle ear infection [13]. Arab herbalists use garlic to treat abdominal pain, infantile colic, diarrhea, diabetes, eye infections, snakebites, dandruff and tuberculosis [14].

Studies have shown that garlic also provides protection to the cardiovascular system by inhibiting platelet aggregation, protecting blood vessels and lipoproteins from damaging effects of free radical oxidation and reducing serum cholesterol levels by inhibiting cholesterol synthesis [15,16]. Experiments have established that, adding fried garlic to feed animals protects them from mutagens [17]. As a commonly used food, garlic and its oils are generally recognized as safe on the Food and Drug Administration (FDA) list [18]. This paper reports on the antibacterial activity of aqueous garlic extract, and the optimal conditions for storage period of this material.

EXPERIMENTAL

Preparation of Extract

Garlic was purchased from the local market in Gaza Strip. The extract was prepared according to the method of Shashikanth *et al.* [19]. The garlic was then washed several times with distilled water. One hundred gram peeled edible portion was chopped and homogenized in 100 ml sterile distilled water in a Waring blender followed by filtration through Whatman No. 1 filter paper. The filtrate was further sterilized by passing through a 22 µm pore-size filter (Millipore, France). The filtrate was collected in a sterile bottle and considered as the aqueous garlic extract with the concentration 1:1. Different concentrations of the extract were prepared (50, 100, 250, 500, 750 and 1000 µg/ml).

Isolation of Bacteria

Ten different pathogenic bacteria (*Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumonia, Streptococcus faecalis, Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis, Pseudomonus aeruginosa* and *Acinetobacter haemolyticus*) were isolated from infected patients in Khan Younis Hospital (Gaza Strip, Palestine) and identified according to published guidelines [20].

Effect of Storage Period and Temperature on the Activity of Aqueous Garlic Extract

Different concentrations (100, 500, 1000 µg/ml) of aqueous garlic homogenates were left in the refrigerator at 2-5 °C for 2, 4, 6, 12, 24, 48 and 72 h before titration. At the end of each period, the antibacterial activity of each concentration was measured.

To study the effect of temperature, similar concentrations of the aqueous garlic extract were exposed to 30, 50, 70, 90 and 100 °C, respectively, for 10 min in a water bath. The extracts were cooled and their antibacterial activity measured.

Calculation of the Inhibitory Concentration

A loopful of inoculum was taken from a pure culture of the respective pathogenic bacteria and inoculated into 5 ml brain-heart infusion broth (Difco). The broth suspension was then incubated aerobically at 37 °C for 18-24 h. The growth so obtained was used as inoculum for the sensitivity assay, where, 0.1 ml from each test organism was added into sterile Petri dish (the inoculum concentration was 10^3 CFU/ml). The required concentration of aqueous garlic extract was added. About 20 ml of molten (45 °C) Muller Hinton agar (Oxoid) was poured into each plate and mixed well. The plates were left until solidification and incubated aerobically at 37 °C for 24 h. The plates were examined and the inhibitory concentration for each bacterium was expressed in viable cells as a percentage of the control in which garlic extract was replaced with sterile distilled water [21].

RESULTS

The effect of storage on the efficacy of the aqueous garlic extract on certain pathogenic bacteria is presented in table 1. The results indicate that, the antibacterial effect of garlic extract increases significantly with storage period at 2-5 °C. The optimal effect was obtained at the period 4-12 h. This effect was obvious in all concentrations but it differed with bacterial kinds. It was observed that, the inhibitory effect of the concentration 1000 µg/ml reached 98-100 % inhibition for all tested bacteria within 6 h. The 6 h storage was therefore considered for all work afterwards. Optimal activity was noted with extract 4-12 h and then activity decreased with storage period, perhaps due to degradation of active ingredients.

Table 1: Effect of storage period on the antibacterial activity (% inhibition) of aqueous garlic extract

Isolates	Concentration (µg/ml)	Storage Period (h)							
		0	2	4	6	12	24	48	72
Escherichia coli	100	7	25	53	47	41	36	0	0
	500	50	60	96	95	89	52	21	8
	1000	73	89	100	100	100	100	100	48
Enterobacter cloacae	100	21	45	69	61	50	24	9	0
	500	34	68	100	100	80	46	20	5
	1000	59	82	100	100	100	100	100	57
Klebsiella pneumonia	100	17	35	53	58	46	32	14	0
	500	44	73	96	100	85	63	25	7
	1000	65	90	100	100	100	100	100	66
Proteus mirabilis	100	13	36	45	58	42	38	18	0
	500	50	63	78	92	94	96	30	9
	1000	62	83	93	100	100	100	95	42
Pseudomonas aeruginosa	100	11	25	44	53	40	34	7	0
	500	44	55	74	88	93	68	31	10
	1000	69	72	90	100	100	100	91	45
Acinetobacter haemolyticus	100	10	23	29	21	14	6	0	0
	500	41	53	70	81	59	31	16	4
	1000	57	70	91	98	88	59	37	10
Staphylococcus aureus	100	18	23	30	35	24	10	0	0
	500	32	66	83	92	76	52	28	8
	1000	53	71	89	100	100	100	96	50
Staphylococcus saprophyticus	100	21	34	42	53	38	21	6	0
	500	49	69	86	96	62	37	22	5
	1000	70	81	86	100	100	100	100	62
Streptococcus faecalis	100	28	43	52	65	48	18	0	0
	500	50	77	86	100	76	52	28	12
	1000	76	83	90	100	100	100	100	80
Streptococcus pneumonia	100	11	32	44	33	21	8	0	0
	500	42	57	86	82	64	43	24	6
	1000	58	84	91	100	98	90	85	35

Storage temperature: 2-5 °C

The results in table 2 illustrate that the optimal storage temperature was at 30-50 °C and antibacterial activity increased by increasing the concentration. For all bacteria, 100% inhibition was obtained at the concentration 1000 µg/ml and 30-50 °C. The potency of all extract concentrations decreased at 70 °C and they all completely lost their activity at 100 °C for 10 min.

Table 2: Effect of storage temperature on antibacterial activity (% inhibition) of aqueous garlic extract

Isolates	Concentration (µg/ml)	Temperature (°C)			
		30	50	70	90
Escherichia coli	100	39	63	21	0
	500	91	96	68	2
	1000	100	100	37	7
Enterobacter cloacae	100	27	70	13	0
	500	86	93	42	0
	1000	100	100	60	3
Klebsiella pneumonia	100	50	83	18	0
	500	69	93	53	2
	1000	100	100	67	12
Proteus mirabilis	100	36	57	23	0
	500	71	86	42	0
	1000	100	100	49	3
Pseudomonas aeruginosa	100	30	64	17	0
	500	73	87	26	0
	1000	100	100	37	4
Acinetobacter haemolyticus	100	28	65	6	0
	500	70	83	22	0
	1000	100	100	25	4
Staphylococcus aureus	100	41	65	0	0
	500	68	88	8	0
	1000	100	100	28	8
Staphylococcus saprophyticus	100	47	81	10	0
	500	73	89	44	0
	1000	100	100	39	3
Streptococcus faecalis	100	62	93	31	0
	500	81	95	34	4
	1000	100	100	57	9
Streptococcus pneumonia	100	34	45	0	0
	500	59	80	0	0
	1000	100	100	18	7

Extraction period: 6 hours

Table 3 presents the antibacterial activity that is proportionally affected by increasing the used concentration. *Streptococcus faecalis* showed the highest inhibitory effect (61%) for the concentration 50 µg/ml and the lowest one (19%) was for *Streptococcus pneumonia*.

At a concentration of 750 µg/ml the inhibitory effect reached 100% on all the tested pathogenic bacteria, except for *A. haemolyticus* and

Streptococcus pneumonia, in which the inhibition was 90% and above.

Table 3: Antibacterial activity (% inhibition) of different concentrations of aqueous garlic extract

Isolates	Concentrations of aqueous garlic extract (µg/ml)					
	50	100	250	500	750	1000
Escherichia coli	51	70	83	96	100	100
Enterobacter cloacae	57	66	78	100	100	100
Klebsiella pneumonia	49	71	84	100	100	100
Proteus mirabilis	42	66	81	94	100	100
Pseudomonas aeruginosa	38	60	70	85	100	100
Acinetobacter haemolyticus	31	47	63	82	90	98
Staphylococcus aureus	32	68	75	92	100	100
Staphylococcus saprophyticus	50	64	83	96	100	100
Streptococcus faecalis	61	83	100	100	100	100
Streptococcus pneumonia	19	51	64	83	92	98

Storage period: 6 hours; Temperature: 50°C

DISCUSSION

Concerning the storage period effect, the results show that, the optimal period to reach the most potent of garlic extract is 6 h. This period can be explained by the fact that allinase enzyme requires about six hours to reach the optimal time to act on alliin. Thereupon, alliin produces the antibacterial material allicin [4]. The efficacy of the aqueous garlic extracts decreases after such period, because allicin changes to entirely different compounds (mainly, diallyl disulfide and diallyl trisulfide). These compounds are volatile and are lost gradually [22].

As observed in this study, the storage temperature of 30-50 °C was suitable for an effective extract against bacteria. This can be explained by the excess of allinase enzyme activity, which is responsible for changing alliin material to allicin at such temperatures. This efficacy disappears at high temperature (70-100 °C) because the main compound alliin or allinase enzyme will be changed or destroyed. Many investigators have reported similar results. For example, the study of Arora and Kaur [22] showed that, the storage of garlic extract or its titrate at refrigeration temperature for 6 days resulted in a 15-29 % loss in activity against bacteria. It loses the activity upon autoclaving. Farbman *et al.* [12], reported that, garlic juice retained its efficacy for three months when stored at -10 °C, whereas, there were no significant effects if garlic is boiled for five minutes before testing. Shashikanth *et al.* [19] stated that, the extract developed higher antibacterial activity than those kept at room temperature after 24 h of incubation at 37 °C. On the other hand, the inhibitory components were completely destroyed or inactivated by autoclaving or heating the extract at 100 °C for 20 min [21, 23]. In this regard, this research suggests that, many of the antimicrobial effects of garlic are reduced or destroyed by heating.

As observed in this study, the antibacterial activity of aqueous garlic extract differs according to the concentration used and the type of the bacteria tested. These results are consistent with many other studies in this respect [24-26].

REFERENCES

[1] A.C. Dutta, A Class-Book of Botany. 16th Ed. Oxford University Press 1984, p 464-465.

[2] C.A. Newall, L.A. Anderson and J.D. Phillipson, Herbal Medicines: A Guide for Health-Care Professionals. London Pharmaceutical Press 1996, 9 p 296.

[3] R. McCaleb, Antioxidant, Antitumor and Cardiovascular Actions of Garlic.

Herbal Gram 29 (1993) 18.

[4] E. Block, Sci. Am. 252 (1985) 114-119.

[5] G. Blania and B. Spangenberg, Planta Med. 57 (1991) 371-375.

[6] P. Josling, Adv. Ther. 18 (2001) 189-193.

[7] C. Stevinson, M.H. Pittler and E. Ernest. Ann. Intern. Med. 133 (2000) 420-429.

[8] C.D. Gardner, L.M. Chatterjee and J.J. Carlson, Atherosclerosis 154 (2001) 213-220.

[9] H. Yoshida, N. Iwata, H. Katsuzaki, R. Naganawa, K. Ishikawa, H. Fukuda, T. Fujino and A. Suzuki, Biosci. Biotechnol. Biochem. 62 (1998) 1014-1017.

[10] G.P. Sivam, J.W. Lampe, B. Ulness, S.R. Swanzy and J.D. Potter, Nutr. Cancer 27 (1997) 118-121.

[11] G.P. Sivam, J. Nutr. 131 (2001) 1106-1108.

[12] K.S. Farbman, E.D. Barnett, G.R. Bolduc and J.O. Klein, Pediatr. Infect. Dis. J. 12 (1993) 613-614.

[13] E.M. Sarrell, A. Mandelberg and H.A. Cohen, Arch Pediatr. Adolesc. Med. 155 (2001) 796-799.

[14] S.M. Abu El-Rob, Medicine and Pharmacy During Ages. Al Ahleea Press, Amman, Jordan. 1991 p 28-29.

[15] S.C. Piscitelli, A.H. Burstein, N. Welden, K.D. Gallicano and J. Falloon, Clin. Infect. Dis. 34 (2002) 234-238.

[16] K.C. Agarwal, Med. Res. Rev. 16 (1996) 111-124.

[17] H.R. Superko and R.M. Krauss, J. Am. Coll. Cardiol. 35 (2000) 321-326.

[18] H. Sumiyoshi, A. Kanezawa and K. Masamoto, J. Toxicol. Sci. 9 (1984) 61-75.

[19] K.N. Shashikanth, S.C. Basappa and V. Sreenivasa Murthy, J. Food. Sci. Technol. 18 (1981) 44-47.

[20] R.W. Burnett, M.H. Haber, E. Hackel, C.A. Hanson, D.F. Keren and E. Lee-Lewandrowski, Clinical Laboratory Medicine. Williams & Wilkins. Philadelphia. 1994, p 1113-1120.

[21] M. Toda, S. Okubo, R. Hiyoshi and T. Shimamura, Lett. Appl. Microbiol. 8 (1989) 123-125.

[22] D.S. Arora and J. Kaur, Int. J. Antimicrobial Agents 12 (1999) 257-262.

[23] K. Song and J.A. Milner, Herbal Gram 56 (2002) 20-21.

[24] M. Yin, H. Chang and S. Tsao. Journal of Food and Drug Analysis. 10 (2002) 120-126.

[25] L. Cellini, E. Di Campli, M. Masulli, S. De Bartolomeo and N. Allocati, FEMS Immunol. Med. Microbiol. 13 (1996) 273-277.

[26] A.Z. Chowdhury, M. Ahsan, S.N. Islam and Z.U. Ahmed. Indian J. Med. Res. 93 (1991) 33-36.