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EFFECT OF STORAGE AND TEMPERATURE OF AQUEOUS GARLIC EXTRACT ON THE GROWTH OF CERTAIN PATHOGENIC BACTERIA

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Abstract: *This research has studied the antibacterial effect of aqueous garlic (Allium sativum) Linn extract on certain pathogenic Gram positive (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 extraction of these materials. The results have revealed that, a concentration of 750 to 1000 µg/ml of the aqueous garlic extract has high antibacterial effect, which reached to 100% with some exceptions. The 6 hrs after extraction and 30-50℃ were found to be the optimal circumstances to reach the optimal efficacy for inhibiting the growth of pathogenic bacteria. Whereas, the temperature of 70-100℃ led to loss of the efficacy of aqueous garlic extract.*
In general, there is high efficacy for aqueous garlic extract against certain pathogenic bacteria and the response was varied with the different bacteria, concentration of the extract, extraction period and temperature.

Key words: *aqueous garlic extract- antibacterial effect- pathogenic bacteria*

INTRODUCTION

A large number of bacterial species have become resistant to antibacterial

drugs. Thus, there is a need to develop alternative strategies. Garlic is known to act as antibiotic and no resistance has been reported for it (Sivam, 2001). Therefor, this research aims to: (a) investigate the antibacterial activity of aqueous garlic extract, (b) determine the optimal conditions for extraction of these materials and (c) study the impact of different temperatures on their effectivity.

Garlic *(Allium sativum)* Linn belongs to the family Liliaceae. This genus contains other kinds such as *A. cepa* L. and *A. porrum* L. The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant (Dutta, 1984).

Numerous studies have indicated that, garlic extract exhibits broad-spectrum antimicrobial activity against many genera of bacteria and fungi at room temperature (Sivam *et al.*, 1997 and Yoshida *et al.*, 1998). Whereas, there are no significant effects if the garlic have been boiled for five minutes before testing (Farbman *et al.*, 1993).

Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium (Newall *et al.*, 1996). 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 (McCaleb, 1993). 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 (Block, 1985). Allicin is further metabolized to vinyldithiines. This breakdown occurs within hours at room temperature and within minutes during cooking (Blania & Spangenberg, 1991).

Since the 1960s it is known that, garlic has a wide useful effect on the human pathogens. Garlic has a long folkloric history as a treatment for colds, coughs, asthma and is commonly stated to strengthen the immune system (Josling, 2001). The potent bulb may also be effective against viruses ranging from the common cold to herpes (Stevinson *et al.*, 2000 and Gardner *et al.*, 2001). Garlic is often combined with the herb mullein in oil products designed to reduce the pain of middle ear infection (Sarrell *et al.*, 2001). Arabic herbalists use garlic to treat abdominal pain, infantile colic, diarrhea, diabetes, eye infections, snake bites, dandruff and tuberculosis (Abu El-Rob, 1991).

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

(Agarwal, 1996 and Piscitelli *et al.*, 2002). Experiments have found that, adding fried garlic to animals' feed protect them from mutagens (Superko & Krauss, 2000).

As a commonly used food, garlic and its oils are generally recognized as safe food on the Food and Drug Administration (FDA) lists (Sumiyoshi *et al.*, 1984).

MATERIALS AND METHODS

Garlic was purchased from the local market (Gaza Strip, Palestine). The extract was prepared according to the method of Shashikanth *et al*. (1981). The garlic was washed several times by distilled water. One hundred gram peeled edible portion was chopped and homogenized in 100 ml sterile distilled water in a Waring blender and 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).

Ten different pathogenic bacteria (*Staph. aureus, Staph. saprophyticus, Strept. pneumonia, Strept. faecalis, E. coli, Enter. cloacae, Kl. pneumonia, Proteus mirabilis, Ps. aeruginosa* and *A. haemolyticus*) were isolated from infected patients in Khan Younis hospital (Gaza Strip, Palestine) and identified according to published guideline (Burnett *et al.*, 1994).

Effect of storage period and temperature on the aqueous garlic extract:

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

To study the effect of temperature, the same mentioned concentrations of the aqueous garlic extract were exposed to 30, 50, 70, 90 and 100°C, respectively, for 10 minutes in water bath. Consequently, the effect of each concentration was measured at each temperature.

Calculation of 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 hrs. 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 hrs. The plates were examined and the inhibitory concentration for each bacteria was expressed in viable cells as a percentage of the control {in which garlic extract was replaced with sterile distilled water} (Toda *et al.*, 1989).

RESULTS

The effect of storage periods on the efficacy of the aqueous garlic extract on certain pathogenic bacteria is presented in Table (1). The results indicated that, the antibacterial effect of garlic extract was increased significantly by augmenting concentrations and the storage period. The optimal effect after extraction is obtained at the period 4-12 hrs. This effect is obvious in all concentrations but it differs with bacterial kinds. It is observed that, the inhibitory effect of the concentration 1000 µg/ml reaches 98-100% for all tested bacteria at the period 6 hrs from extraction. The 6 hrs after extraction is therefore considered for all work afterwards. Whereas, the aqueous garlic extracts at the concentration 100 µg/ml were stable for 24 hrs and sometimes 48 hrs after extraction and no large changes occurred in their efficacy. Also, there is clear stability for the concentrations of 500 and 1000 µg/ml and reached 48-72 hrs.

For *Strep. faecalis,* the concentration of 1000 µg/ml shows the higher antibacterial effect (80%) after 72 hrs of extraction. Whereas, the lowest effect (10%) is for *A. haemolyticus* at the same period and concentration.

The results in Table (2) illustrated that, the optimal antibacterial effect was at 30-50°C and increase by increasing the concentration.

However, 100% of the tested bacteria were inhibited at the concentration 1000 µg/ml and temperature 30-50°C. Whereas, the potency of all concentrations decreases at 70°C and completely lose their effectivity at 100°C for 10 minutes.

Table (3) presents the antibacterial effect that is proportionally affected by increasing the used concentration. *Strept. faeclis* shows the higher inhibitory effect (61%) for the concentration 50 µg/ml and the lowest one (19%) is for *Strept. pneumonia*. While, the inhibitory effect for the concentrations 750 and 1000 µg/ml reaches to 100% on the tested pathogenic bacteria, except

Strept. pneumonia and *A. haemolyticus*, in which, the inhibition reaches 90% and 92%, respectively, for the first concentration. Whereas, for the second concentration it reaches 98% for both isolates.

Table (1): The effect of storage period of aqueous garlic extract efficacy on certain bacteria.

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

Strept.	500	42	57	86	82	64	43	24	6
pneumonia	1000	58	84	91	100	98	90	85	35

*Conc.= concentration

Table (2): The effect of different temperatures on the efficacy of aqueous garlic extract on certain bacteria.

Isolates	**Conc. µg/ml**	**Temperature ºC**				
		30	**50**	**70**	**90**	**100**
		% of inhibition				
E. coli	100	39	63	21	0	0
	500	91	96	68	2	0
	1000	100	100	37	7	0
Enter. cloacae	100	27	70	13	0	0
	500	86	93	42	0	0
	1000	100	100	60	3	0
Kl. pneumonia	100	50	83	18	0	0
	500	69	93	53	2	0
	1000	100	100	67	12	0
Proteus mirabilis	100	36	57	23	0	0
	500	71	86	42	0	0
	1000	100	100	49	3	0
Ps. aeruginosa	100	30	64	17	0	0
	500	73	87	26	0	0
	1000	100	100	37	4	0
A. haemolyticus	100	28	65	6	0	0
	500	70	83	22	0	0
	1000	100	100	25	4	0
Staph. aureus	100	41	65	0	0	0
	500	68	88	8	0	0
	1000	100	100	28	8	0
Staph. saprophyticus	100	47	81	10	0	0
	500	73	89	44	0	0
	1000	100	100	39	3	0
Strept. faecalis	100	62	93	31	0	0
	500	81	95	34	4	0
	1000	100	100	57	9	0

	100	34	45	0	0	0
Strept. pneumonia	500	59	80	0	0	0
	1000	100	100	18	7	0

Table (3): The effect of different aqueous garlic extract concentrations on certain bacteria.

Isolates	**Concentrations of aqueous garlic extract (µg/ml)**					
	50	100	250	500	750	1000
	% of inhibition					
E. coli	51	70	83	96	100	100
Enter. cloacae	57	66	78	100	100	100
Kl. pneumonia	49	71	84	100	100	100
Proteus mirabilis	42	66	81	94	100	100
Ps. aeruginosa	38	60	70	85	100	100
A. haemolyticus	31	47	63	82	90	98
Staph. aureus	32	68	75	92	100	100
Staph. saprophyticus	50	64	83	96	100	100
Strept. faecalis	61	83	100	100	100	100
Strept. pneumonia	19	51	64	83	92	98

DISCUSSION

The importance of this study lies in its contribution to state many facts and prove that garlic, which is readily available and widely used, contains effective materials with the ability to inhibit the growth of certain pathogenic bacteria. In addition, it finds the optimal circumstances for extraction of these effective materials and measures the temperature effect on bacterial inhibition.

Concerning the storage period effect, the results show that, the optimal period to reach the most potent of garlic extract after extraction is six hrs.

This period can be explained by the fact that Allinase enzyme requires about six hrs to reach the optimal time to act on Alliin. Thereupon, Alliin produces the antibacterial material Allicin (Block, 1985). The efficacy of the aqueous garlic extracts decrease after such period, because Allicin changes to entirely different compounds (mainly, diallyl disulfide and diallyl trisulfide). These compounds are volatile with rate according to their components, molecular weights and temperature of exposure, where part of the effective materials is lost gradually (Arora & Kaur, 1999). This indicates that, the constant of the effective materials is more in low temperature.

As observed in this study, the temperature of 30-50°C is suitable for increasing the efficacy of the extract against bacteria. This can be explained by the excess of Allinase enzyme activity which responsible of changing Alliin material to Allicin at such temperatures. This efficacy disappears at high temperature (70-100°C), because the main in-effected compound Alliin or Allinase enzyme will be changed or destructed. Consequently, the construction of the effective materials declines or stops. Many investigators have reported quite similar results. For example, the study of Arora & Kaur (1999) showed that, the storage of garlic extract or its filtration at refrigeration temperature for 6 days resulted in a 15-29% loss in activity against bacteria. Whereas, garlic extract completely lose the activity upon autoclaving. Farbman *et al*. (1993) also, reported that, the effective materials still protect their efficacy for three months after storing garlic juice at –10°C. Whereas, there were no significant effects if garlic is boiled for five minutes before testing.

Shashikanth *et al*. (1981) stated that, the extract developed higher antibacterial activity after 24 hrs of incubation at 37°C than those kept at room temperature. On the other hands, the inhibitory components were completely destroyed or inactivated by autoclaving or heating the extract at 100°C for 20 minutes (Toda *et al.*, 1989 and Song & Milner, 2002).

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 potentiality of aqueous garlic extract differs according to the used concentration and the type of the tested bacteria. These results are consistent with many other studies in this respect (Cellini *et al*., 1996 and Yin *et al*., 2002). For example, Cellini *et al*. (1996) demonstrated that, the concentration of 5mg/ml of aqueous garlic extract inhibited 90% of 16 clinical isolates. Chowdhury *et al.* (1991) also, found that, 5 µl/ml of garlic extract was the minimum inhibitory concentration to antibiotic-resistant bacteria.

Finally, it is important to emphasize that, the aqueous garlic extract poses

the advantages of easily preparation and low cost. Therefor, garlic consumption may be used as an economic way for patients or hospital workers to prevent infections and decrease the problem of multi-drug resistance in Palestine.

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