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Effect of garlic extract on human intestinal microflora

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SUMMARY

Influence of aqueous extract of five garlic genotypes (HG-1, HG-6, HG-17, HG-19, G-1) was examined on the population of *Salmonella typhimurium, Staphylococcus aureus, Enterococcus faecalis and Escherichia coli.* Monitoring of the growth after 6h and 24h revealed that garlic extract was effective in checking the growth of these pathogens. The aqueous extract of garlic variety HG-19 bulbs showed the maximum inhibition against all the microorganisms tested. The best garlic extract concentration emerged to be 1.5% that produced more than 90% growth inhibition.

Key words : Garlic, Intestinal microflora, Growth inhibition, Colony forming units, Allicin

Plants accumulate high concentrations of different chemicals that constitute the basis for modern drug equivalents (Srivastava *et al.,* 1982). The plants of the genus Allium, having anti-microbial activity and widely used in the human diet, are garlic and onion (AI-Dalaimy and Ali, 1970). Garlic is the second most widely cultivated allium used for home consumption in foods, pharmaceutical preparations and for earning foreign exchange. Garlic sap inhibits the growth of several gram positive and gram-negative food spoilage and pathogenic bacteria including *Escherichia, Enterobacter, Salmonella, Shigella* and *Pseudomonas*. Aqueous extract of garlic contains the water soluble thiosulfinates (allicin etc.) and therefore have strong antimicrobial activity (AI-Dalaimy and Ali,1970; Augusti,1996; Thompkinson and Singh, 2000). The use of powder from fresh garlic was proved to be much effective than that from one year old garlic against *Escherichia coli* and 1% solution of fresh garlic powder eradicated the *E. coli* O-157 in 6h (Sasaki *et al.,* 1999). The antimicrobial activity was resistant to heat treatment of 100^{O}C for 20 min. Garlic therapy has also been suggested in flatulence, constipation, faulty digestion, inadequate food intake, chronic coughs, leprosy and in many other diseases (AI-Dalaimy and Ali, 1970; Augusti, 1996; Thompkinson and Singh, 2000; Sasaki *et al.,* 1999).

Keeping in view the variegated facts and tangible uses of garlic, the present study was designed to examine the effect of aqueous garlic extract of five varieties on some of the microorganisms commonly populated in the human intestine.

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MATERIALS AND METHODS

Plant materials:

Five garlic varieties (HG-1, HG-6, HG-17, HG-19, G-1) were grown in a randomized block design in three replicates at the Vegetable Research Farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar. The recommended doses of fertilizers and other agronomical practices were adopted to raise the crop as per package and practices of the university (HAU, 1981). Garlic bulbs were collected at 195 days after sowing.

Bacterial cultures:

The microbial cultures of *Escherichia coli* and *Salmonella typhimurium* were obtained from Department of Veterinary Microbiology, CCS HAU, Hisar and of *Staphylococcus aureus* and *Enterococcus faecalis* from Department of Microbiology and Biotechnology, SBS (PG) Institute of Biomedical Sciences, Balawala, Dehradun. All the bacterial cultures were maintained on nutrient agar slants at 4^{O}C with repeatedly transfer on fresh slants after one month.

Preparation of garlic extract:

Aqueous garlic extract was prepared by crushing 1g of garlic bulbs with 100 ml of distilled water in a pestle and mortar and filtered through Millipore filter (0.45 µm) and stored in presterilized 250 ml conical flask. Periodically 0.5, 1.0, 1.5 or 2.0 ml of garlic extracts was drawn aseptically and added to the nutrient broth.

Procedure for antibacterial activity:

Antibacterial activity of aqueous garlic extract was

determined by measuring the growth (by viable plate counting) in presence of different concentrations of garlic extract. Twenty four h old slant culture was inoculated into nutrient broth and incubated for 24h at 37^{O}C in the incubator. Equal biomass was inoculated (based on the absorbance of the culture at 600) into fresh nutrient broth tubes containing different concentrations of garlic extract of HG-1, HG-6, HG-17, HG-19, G-1 varieties. The tubes were incubated at 37^{O}C. Samples were withdrawn at an interval of 6h and 24h and plated on nutrient agar after appropriate dilution and the plates were incubated at 37^{O}C for 24h and colony forming units (CFU) were counted with the help of colony counter. Control was also set up in which no garlic extract was added.

RESULTS AND DISCUSSION

Different garlic varieties responded differently with respect to their affect on *Salmonella typhimurium* (Table 1). At 0.5% concentration of the extract, significant inhibition was observed in all the varieties after 6h of the incubation (43.3% in HG-1, 41.7% in HG-6, 43.2% in HG-17, 43.6% in HG-19 and 39.9% in G-1); and after 24h of incubation, inhibition was reduced considerably, to about half (17.9% in HG-1, 14.3% in HG-6, 18.3% in HG-17, 21.8% in HG-19 and 10.3% in G-1). At 1% level, inhibition of *Salmonella typhimurium* growth was higher than at 0.5% level after 6h interval; however, after 24h interval, the growth of this organism was highly reduced amounting to an average value of 92.8%. Still higher concentrations of garlic extract (1.5-2.0%) resulted in drastic growth inhibition of *S. typhimurium*, amounting to the range of 87.7 to 99.7%, after 6h and 24h of incubation.

The data in Table 2 shows that at 0.5 per cent concentration of garlic extract, the per cent growth inhibition of *Enterococcus faecalis* was 63.4% by HG-1, 62.9% by HG-6, 63.2% by HG-17, 65.2% by HG-19 and 60.7% by G-1 after 6h of incubation. However, after

Table 1 : Effect of garlic bulb aqueous extract on *Salmonella typhimurium* population

Concentration (%)	0 h	Number of organism (x10^5) at the end of 6 and 24 h									
		6h					24h				
		HG-1	HG-6	HG-17	HG-19	G-1	HG-1	HG-6	HG-17	HG-19	G-1
0	13	3815	3646	3966	3715	4017	25625	24415	24620	26714	25908
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
0.5	13	2163	2124	2253	2096	2413	21028	20912	20108	20896	23236
		(43.3)	(41.7)	(43.2)	(43.6)	(39.9)	(17.19)	(14.3)	(18.3)	(21.8)	(10.3)
1.0	13	1329	1411	1385	1103	1585	1781	1872	1710	1628	2069
		(65.2)	(61.3)	(65.0)	(70.3)	(60.5)	(93.0)	(92.3)	(93.0)	(93.9)	(92.0)
1.5	13	368	427	389	245	494	541	614	539	394	709
		(90.3)	(88.3)	(90.2)	(93.4)	(87.7)	(97.9)	(97.5)	(97.8)	(98.6)	(97.3)
2.0	13	195	256	210	95	307	211	293	203	94	323
		(94.9)	(92.9)	(94.7)	(97.4)	(92.3)	(99.2)	(98.8)	(99.2)	(99.7)	(98.8)

Values in parenthesis indicate per cent inhibition

Table 2: Effect of garlic bulb aqueous extract on *Enterococcus faecalis* population

Concentration (%)	0 h	Number of organism (x10^5) at the end of 6 and 24 h									
		6h					24h				
		HG-1	HG-6	HG-17	HG-19	G-1	HG-1	HG-6	HG-17	HG-19	G-1
0	9	3893	3785	3805	3915	3634	7580	7919	7746	7240	7476
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
0.5	9	1442	1403	1398	1363	1426	3863	4146	3982	3523	3914
		(63.4)	(62.9)	(63.2)	(65.2)	(60.7)	(49.0)	(47.6)	(48.6)	(51.3)	(47.6)
1.0	9	831	847	820	746	883	2384	2610	2468	2385	2495
		(78.6)	(77.6)	(78.5)	(80.9)	(75.7)	(68.5)	(67.0)	(68.1)	(67.1)	(66.6)
1.5	9	16	19	14	11	21	36	40	37	34	39
		(99.5)	(99.5)	(99.6)	(99.7)	(99.4)	(99.5)	(99.5)	(99.5)	(99.5)	(99.4)
2.0	9	11	13	10	7	15	24	31	26	20	32
		(99.7)	(99.6)	(99.7)	(99.8)	(99.6)	(99.7)	(99.6)	(99.6)	(99.7)	(99.5)

Values in parenthesis indicate per cent inhibition

24h of incubation with identical concentration, the per cent inhibition was slightly decreased. However, with increase in concentration of garlic extract (1, 1.5 and 2.0%), level of the inhibition was significantly increased to the maximum of approximately 99.7%.

Similarly, a drastic reduction of growth of *Staphylococcus aureus* after 6h as well as 24h of incubation was recorded (90.2%) at low concentration (0.5%) of aqueous extract of all the garlic genotypes (Table 3). Further increase in the concentration of garlic extract, resulted gradual decrease of growth to still lower levels.

Effect of garlic extract at 0.5% concentration against *E. coli* revealed that the comparative growth inhibition was lower after 6h and 24h (Table 4). However, with increase in concentration of garlic extract of all the varieties from 0.5-1%, a significant growth inhibition 90.9-98.7% was observed. A slight increase in inhibition was observed beyond 1% level. HG-19 exhibited maximum growth inhibition against all the organisms included in the study at varying concentration of garlic extract followed by HG-1 and HG-17, while HG-6 and G-1 showed minimum growth inhibition. The best garlic concentration emerged to be 1.5% that produced more than 90% of growth inhibition for *Salmonella typhimurium*, *Enterococcus faecalis* and *Staphylococcus aureus* but for *E. coli*, 1.0% concentration of the garlic extract produced the same effect.

The decrease in counts of the microorganisms tested by different concentrations of garlic extract after 6h and 24h of incubation reflects the antimicrobial potency of the aqueous garlic extract. The probable reasons for differential behavior of the extract against single organism tested may be due to varied content of active principle, reducing agents namely cystine, glutathione; and of the same aqueous extract against different organisms presently tried, might be due to the reason that they were not equally sensitive to garlic extract (Augusti,1990). The antimicrobial

Table 3: Effect of garlic bulb aqueous extract on *Staphylococcus aureus* population

Concentration (%)	0 h	Number of organism ($x10^5$) at the end of 6 and 24 h									
		6h					24h				
		HG-1	HG-6	HG-17	HG-19	G-1	HG-1	HG-6	HG-17	HG-19	G-1
0	15	2404	2517	2499	2310	2711	6000	24415	5996	5698	6229
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
0.5	15	405	524	436	321	488	713	20912	742	558	803
		(83.2)	(79.2)	(82.5)	(86.1)	(82.0)	(88.1)	(14.3)	(87.6)	(90.2)	(87.1)
1.0	15	286	413	296	193	391	661	774?	701	476	781
		(88.1)	(83.6)	(88.2)	(91.6)	(85.6)	(89.0)	(87.9)	(88.3)	(91.6)	(87.5)
1.5	15	154	263	194	90	260	387	486	412	208	474
		(93.6)	(89.5)	(92.2)	(96.1)	(90.4)	(93.5)	(92.4)	(93.1)	(96.3)	(92.4)
2.0	15	85	176	118	40	183	210	294	223	87	290
		(96.5)	(93.0)	(95.3)	(98.3)	(93.2)	(96.5)	(95.4)	(96.3)	(98.5)	(95.3)

Values in parenthesis indicate per cent inhibition.

Table 4: Effect of garlic bulb aqueous extract on *Escherichia coli* population

Concentration (%)	0 h	Number of organism ($x10^5$) at the end of 6 and 24 h									
		6h					24h				
		HG-1	HG-6	HG-17	HG-19	G-1	HG-1	HG-6	HG-17	HG-19	G-1
0	4	756	805	726	795	704	3000	2895	2950	2900	3150
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
0.5	4	568	674	530	560	564	2117	2156	2050	558	803
		(24.9)	(16.3)	(26.9)	(29.5)	(19.9)	(27.6)	(25.5)	(30.5)	(34.8)	(22.2)
1.0	4	14	45	19	10	27	273	316	229	210	398
		(98.1)	(94.4)	(97.4)	(98.7)	(96.2)	(90.9)	(89.1)	(92.2)	(92.7)	(87.4)
1.5	4	13	42	16	8	25	205	287	140	135	318
		(98.3)	(94.8)	(97.8)	(98.9)	(96.4)	(93.2)	(90.1)	(95.2)	(95.3)	(89.9)
2.0	4	9	31	11	3	19	127	214	95	64	225
		(98.8)	(96.1)	(98.5)	(99.6)	(97.3)	(95.8)	(92.6)	(96.8)	(97.8)	(92.8)

Values in parenthesis indicate per cent inhibition

activity of garlic extract covers wide spectrum of organisms (Subrahmanyan *et al.*,1958; Sharma *et al.*, 1977; Kumar and Sharma, 1982; Sreenivasa Murty *et al.*, 1982; Augusti,1990; Groppo *et al.*, 2007) . The garlic extract produces both bactericidal and bacteriostatic effects on the organisms tested *in vitro* (Kumar and Sharma, 1982; Sreenivasa Murty *et al.*, 1982; Augusti, 1990). Groppo *et al.* (2007) evaluated the antimicrobial activity of two garlic clones crude extracts against oral streptococci *in vitro* and *in vivo*. They concluded that the garlic clones have antimicrobial properties *in vitro* against streptococci and anticarcinogenic properties against oral microorganisms in spite of its adverse effects. Reduction in effectiveness of the garlic extract upon prolong incubation may be due to inactivation of the active principle, loss during storage, as the active principle being volatile, appearance of new microbial agent (mutant) which are more resistant against the garlic extract, and interaction of the active principle with medium components (Barone and Tansey, 1977; Shashikanth *et al.*, 1981). The exact mechanism of action of garlic as antimicrobial agent is not well understood. Wills (1956), opined that allicin, the main active principle of the garlic, affects the activity of important metabolic enzymes especially those having –SH groups. Groppo *et al.* (2007) reported that the garlic extract inhibits the protein synthesis in microbes and this thinking was earlier held by George *et al.* (1973)

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