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Cellular Effects of Garlic (*Allium sativum*) Extract on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract: Effects of garlic extract at 67, 134 and 201 mg mL^{-1} on *Pseudomonas aeruginosa* and *Staphylococcus aureus* were studied. In the absence of the extract, the cells grew to high densities within 11/2 h at 37°C. Garlic extract-treated cells reduced in number and died. Percentage living cells at 201 mg mL^{-1} was 0% for both bacteria. Sucrose and $MgSO_4$ stabilized and protected the cells. At 67, 134 and 201 mg mL^{-1} of the extract in the presence of this sugar and the compound, 47, 4 and 0% of *Ps. aeruginosa* cells were viable. Microscopic examination of carbol fuschin and Giemsa stained cells showed that the garlic treated cells were bigger in size than those of untreated ones; and intact and definite nuclei were lacking. The cell wall was the target of attack and the extract was bacteriolytic in action.

Key words: Garlic extract, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

INTRODUCTION

Garlic (*Allium sativum*) is a bulbous, perennial, medicinal plant which belongs to the family Liliaceae. It has inhibitory effects against many microbes (Banerjee and Maulik, 2002). The antimicrobial activity of this plant has been known for a long time. In the middle ages, the plant was used by French priests against bubonic plaque, a bacterial infection (Heinach and Larry, 1996). During the World War 1, the European soldiers used garlic to prevent infection of their wounds. Today, many research works have shown that garlic is effective against bacteria such as *Helicobacter pylori*, *Shigella dysenteriae*, *Sh. flexneri*, *Sh. sonnei*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pr. mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Salmonella typhimurium*, *Sal. paratyphi* B and C, *Vibrio cholerae*, *Corynebacterium diphtheriae* and *Streptococcus faecalis* (Chowdhury *et al*., 1991; Anesini and Perez, 1993; Cellini *et al*., 1996; O'Gara *et al*., 2000; De *et al*., 2001; Ross *et al*., 2001; Barnes *et al*., 2002; Boboye and Dayo-Owoyemi, 2004; Calsamiglia *et al*., 2007).

Garlic has antifungal activity against *Cryptococcus neoformans*, thus it is used in the treatment of cryptococcal meningitis (Davis *et al*., 1990). Venugopal and Venugopal (1995) showed that garlic could be an effective antidermatophytic agent. It is equally antagonistic against other fungi including *Aspergillus flavus*, *Candida albicans*, *Alternaria* species, *Rhodotorula* and *Torulopsis* (Arora and Kaur, 1999; Jellin *et al*., 2000; Martin and Ernst, 2003; Lemar *et al*., 2005).

The antiviral action of garlic is directed towards the viruses of Herpes simplex 1 and 2 (ASV I and II), Vesicular stomatitis, Vaccinia, Para-influenza and Acquired Immune Deficiency Syndrome (AIDS) (Weber *et al*., 1992; United States Department of Agriculture, 2003). Rajabather (1994) reported immune boosting ability of garlic when administered with viral vaccine in AIDS condition. Garlic has also been shown to possess antiparasitic effect mainly against protozoan parasites including *Trypanosoma* strains, *Entamoeba histolytica*, *Giardia lamblia* and *Hymenolepsis nana* (Lun *et al*., 1974; Suffar and Mokhtar, 1991; Nok *et al*., 1996; Ross, 1999).

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Some microorganisms have become resistant to certain antibiotics. Garlic can be used on microorganisms that have particularly developed resistant to antibiotics. This can be seen in the study of Tsao and Yin (2001) who explained that garlic oil and four diallyl sulphides showed *in vitro* activity against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. It is known that garlic extract uses different mechanisms to antagonize various living systems. It is important to examine these modes of action to enable appropriate application of the plant for use particularly in cases where antibiotics are disallowed for use such as in rumen fermentation. The use of antibiotics in animal feeds is facing reduced social acceptance and their use has been banned in the European Union since January, 2006. For this reason, scientists have become interested in evaluating other alternatives to control specific microbial populations to modulate rumen fermentation. It was shown that the ability of garlic components to decrease methane production in rumen fermentation helps to improve the efficiency of energy use in the rumen (Busquet *et al*., 2005). Earlier to this study we observed that garlic, ginger, onion, basil, hot and sweet peppers inhibited the growth of *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa* and *Escherichia coli*. We also found out that garlic was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at minimum inhibitory concentrations of 161 and 134 mg mL^{-1} respectively (Boboye and Dayo-Owoyemi, 2004). In order to know the means by which garlic acts on *Staph. aureus* and *Ps. aeruginosa*, we treated cells of these bacteria with garlic extracts and discussed here that garlic possesses cellular effects on *Staph. aureus* and *Ps. aeruginosa*.

MATERIALS AND METHODS

Materials and Bacterial Cultures

This research was carried out in the Department of Microbiology, Federal University of Technology, Akure, Nigeria. The work was completed just before its submission in the year, 2007. Garlic (*Allium sativum*) was obtained from "Oba" and "Bodija" markets in Akure and Ibadan, Nigeria. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were provided by the University Teaching College, Ibadan, Nigeria. They were grown at 37°C for 24 h and stored on nutrient agar. The nutrient agar and its broth were purchased from Lab M, Topley, England. Garlic extract was prepared in sterile distilled water as described by Boboye and Dayo-Owoyemi (2004).

Determination of Mode of Action of Garlic

Preparation of the Cultures

Method of Park (1982) was used with little modification to determine the mode of action of the garlic at 67, 134 and 201 mg mL^{-1}. A set of eleven tubes received additions as written in Table 1. An 18 h old culture at OD_{670} of 0.635 was used. Tubes 1, 2(a,b,c), 3 and 4(a,b,c) were incubated at 37°C for 11/2 h and 5(a,b,c) for 6 h.

Table 1: Contents of test tubes used for *in vitro* study on mode of action of garlic on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

	1	2a	2b	2c	3	4a	4b	4c	5a	5b	5c
Double strength nutrient broth (mL)	4	4	4	4	4	4	4	4	4	4	4
2 M sucrose (mL)	0	0	0	0	2	2	2	2	0	0	0
0.1 M $MgSO_4$ (mL)	0	1	1	1	0	1	1	1	0	0	0
Garlic extract (0.67 g mL^{-1}) (mL)	0	1	0	0	0	1	0	0	1	0	0
Garlic extract (1.34 g mL^{-1}) (mL)	0	0	1	0	0	0	1	0	0	1	0
Garlic extract (2.01 g mL^{-1}) (mL)	0	0	0	1	0	0	0	1	0	0	1
Demineralized water (mL)	4	2	2	2	2	0	0	0	3	3	3
Culture (mL)	2	2	2	2	2	2	2	2	2	2	2

Determination of Growth of the Bacteria

Turbidity of the cultures in the tubes were read at 670 nm. Cells were stained with methylene blue on a glass slide and examined microscopically at x40. Living and dead cells were counted. An aliquot (1 mL) of the *Staphylococcus aureus* culture was pour-plated in nutrient agar and incubated as before in order to determine its viability since the bacterium is not motile (it is not possible to see its motility microscopically).

Test for Effect of Garlic Extract on Cell Wall

Formalin (0.2 mL) was added to each tube, left for 5 min and 2 mL of the content was centrifuged at $12,168\times10^3$ g (MSE Minor 35 Centrifuge) for 15 min. The cells in tubes 1-4 were resuspended in 0.1 mL demineralised water. Smears were made on glass slides, dried and stained with dilute carbol fuschin for 30 sec, rinsed in water, air-dried, examined under the microscope and photomicrographs were taken at x400.

Acid-Giemsa Staining Method of DNA

Dense smear of formalin treated cells in each of the tubes labelled 5 were made on a coverslip, air-dried and heated in 1 M HCl at 55°C for 10 min. The coverslip was rinsed in tap water for 30 sec. Three millilitres of dilute Giemsa dye was added to a slide and the smeared coverslip was turned upside down to dip the smear in Giemsa stain for 30 min. It was rinsed in water and blotted dry. Vaseline was applied to edges of the coverslip and placed on a slide. It was observed under oil immersion lens and photomicrographs were taken.

RESULTS AND DISCUSSION

All the cells in tubes 1 and 3 were living (Table 2) because there was no garlic extract added to them. Number of living cells in tubes 2, 4 and 5 reduced. Viable count of *Staph. aureus* also showed a similar effect with no considerable viable cells in the third plates of each set of the tubes (Table 2). This was due to action of garlic extract on the bacteria as it has been reported by many scientists including Groppo *et al*. (2002) who found that levels of some microorganisms were reduced after treatment with garlic and tea tree oil separately. Higher number of living cells were observed in tubes 2 and 4 than 5 due to the effect of sucrose and $MgSO_4$ added which were lacking in tubes of number 5 treatment. The sucrose used was meant to protect the cells from lysis and magnesium sulphate stabilizes unprotected membranes (Park, 1982). The difference in percentage of viable cells and turbidity of culture in the tubes indicates effect of increase in concentration of the extract. This pattern

Table 2: Effect of garlic extract on the growth and viability of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

	Optical density (670 nm)		Viable cells (%)	
Tubes	*Ps. aeruginosa*	*Staph. aureus*	*Ps. aeruginosa*	*Staph. aureus*
1	0.730	0.368	100	100
2a	0.615	0.318	39	19
2b	0.415	0.290	2	10
2c	0.349	0.203	0	0
3	0.840	0.457	100	100
4a	0.329	0.186	47	25
4b	0.210	0.105	4	3
4c	0.100	0.055	0	0
5a	0.442	0.218	7	4
5b	0.189	0.139	3	1
5c	0.050	0.039	0	0

Ps. aeruginosa: *Pseudomonas aeruginosa* and *Staph. aureus*: *Staphylococcus aureus*, Treatments 1, 2a, 2b, 2c, 3, 4a, 4b, 4c, 5a, 5b and 5c: are as explained in the method

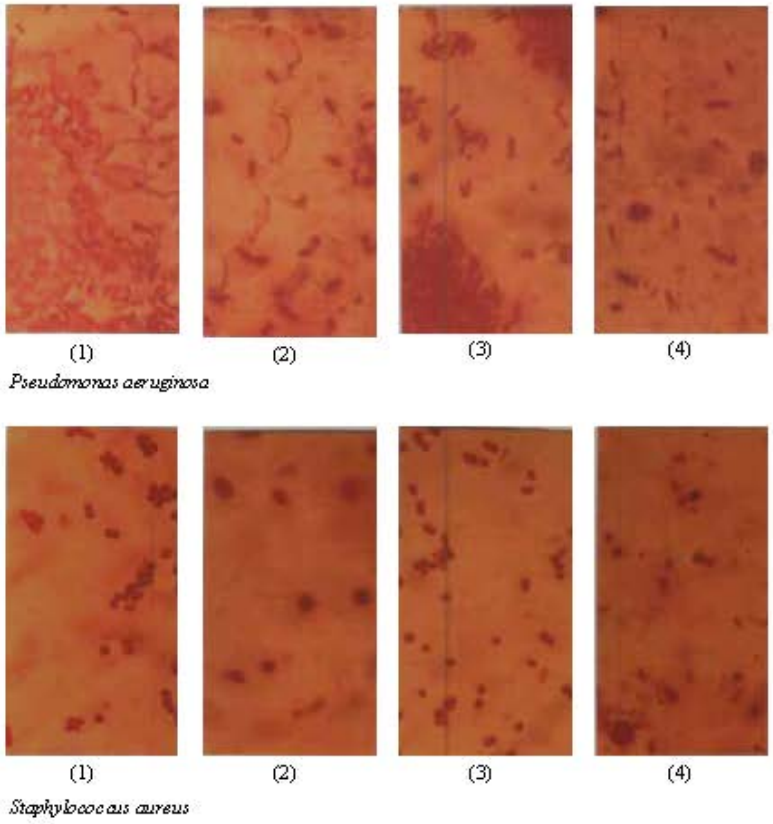

Fig. 1a: Monograph of bacterial cells treated and untreated with garlic extract. 1, 2, 3 and 4 are number of tubes as recorded in method

of the effect of the garlic is common to the two bacteria used in this experiment. This data is analogous to the dose-dependent result obtained from the time course viability studies and microscopy carried out on *H. pylori* treated with undiluted garlic oil (16 to 32 μg mL^{-1}), garlic powder (250 to 500 μg mL^{-1}) and allicin (4.0 μg mL^{-1}) after a lag phase of about 1 to 2 h (O'Gara *et al*., 2000).

Figure 1a shows that the cells in tubes 1 and 3 have intact cell walls. Cells in other tubes (2 and 4) appeared bigger than those in tubes 1 and 3 while those in 5 have lysed. Monographic pictures of DNA stained bacterial cells showed irregular distribution of cellular particles (Fig. 1b). This indicates that cell division and DNA replication have occurred in the cells. Abnormal elongation of cells was not observed. This is because essential oils of plants including that of garlic can interact with microbial cells membranes and inhibit the growth of some gram-positive and gram-negative bacteria (Calsamiglia *et al*., 2007). *Allium sativum* reduced chronic *Helicobacter pylori* disease at minimum bactericidal concentrations (O'Gara *et al*., 2000); confirming the killing potential of the garlic extract on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Feldberg *et al*. (1988) reported that there was partial inhibition of DNA and protein synthesis by garlic extract. Clear nuclei were not observed in this work because the cellular contents were released outside the cells (due to cell lysis) and scattered in the medium and thus they were observed as particles in the monographic pictures. Bacteriolytic agents like this garlic extract bind tightly to cellular target of the relevant microorganisms and induce killing by lysis which was observed as a decrease in cell numbers or in turbidity after the agent is added. They inhibit cell membrane and cell wall synthesis (Brock *et al*., 1984).

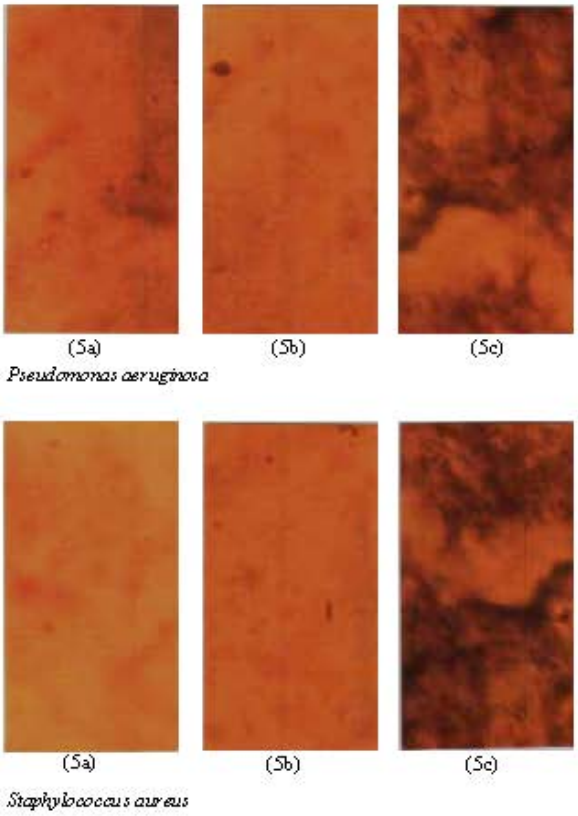

Fig. 1b: Photomicrograph of cells stained with Giemsa dye. 1, 2, 3 and 4 are number of tubes as recorded in method

The mode of action of garlic appeared to differ from one organism to the other. In a study carried out by Israeli researchers, Mercola (1997) reported that allicin from garlic blocks the action of bacterial enzymes by reacting with thiols thereby inhibiting the growth of the microbe. Garlic extract can also cause death of microorganisms through oxidative stress as was demonstrated in *Candida albicans* with concomitant inhibition of both growth and respiration of the yeast (Lemar *et al*., 2005).

This study has shown that garlic extract is antagonistic to *Pseudomonas aeruginosa* and *Staphylococcus aureus* by causing their cells to rupture. More experiments should be considered to investigate the detail steps involved in the mechanism of action.

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