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The Morphological and Biochemical Alterations Induced by *Allium sativum* in Human Microbial Pathogens

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INTRODUCTION

Allium sativum commonly known as garlic is being used as herbal medicine for treatment of cardiovascular diseases(Farnsworth,1988). Recently several herbal extracts have been tried as anti microbials (Elizabeth, 2001; Haraguchi et al., 1999). The minimal inhibitory concentrations of *A. sativum* on *Y. enterocolitica, S. typhi, S. typhimurium* and *S. aureus* have been determined (Elizabeth, 2001) as 10mg ml^{-1}, 8mg, 7.5mg, and 8mg respectively against $1x10^9$ cells of each culture. So far there were no reports on the biochemical alterations induced by sub inhibitory concentrations of *A. sativum,* in human microbial pathogens. Therefore the aim of the present investigation was to study the morphological and biochemical alterations induced by sub inhibitory concentrations of *A. sativum* on human pathogenic microorganisms.

MATERIALS AND METHODS

All the microbial cultures were obtained from MTCC, Chandigarh, India. *Allium sativum* was obtained from local market, pealed off, ground to a fine paste in a grinder. Garlic stock solution was prepared by dissolving fixed amount of the paste in sterile distilled water.

Bacterial Culture Conditions: To study the morphological alterations, a single colony of each type of culture viz. *S. typhimurium*, *S. typhi*, and *S. aureus* was inoculated in 5ml of nutrient broth (0.5% peptone, 0.3% beef extract, and 0.5% NaCl) and incubated at 37°C for 18-24h. This primary culture was used to inoculate the secondary broth cultures with $1x10^9$ cells/ml and the sub inhibitory concentration of garlic used was 7mg ml^{-1}. The control did not receive the herbal medicine. These secondary cultures were incubated at 37°C for 18h-24h. Gram staining and Hanging drop methods (Cappuccino and Sherman, 1999) were performed with secondary broth cultures and observed under oil immersion objective. To study the alterations in the colony morphology, secondary cultures of both control and experimental were plated on nutrient agar plates using serial dilution method and incubated at 37°C for 18-24h and observed. Similarly *Y. enterocolitica* was cultured in brain heart infusion broth or agar (Elizabeth, 2001). Briefly describing, the gram staining was done by preparing the smear of culture on a glass slide, and flooding with crystal violet, Gram's iodine, ethyl alcohol, and safranine for 30-45 sec. smear was washed with water after each of these steps. Later it was blotted, and oil drop was placed and observed under oil immersion objective. For hanging drop method a depression slide was used and a hanging drop of each culture was made from the cover slip and observed under oil immersion objective. Fermentation reactions were carried out to study acid and gas formation. The peptone water was inoculated with $1x10^9$ cells ml^{-1} of each culture type to which specific (10%) sugars such as lactose, glucose, dextrose, sucrose and mannitol (High Media, Mumbai, India) were added. To test the gas formation a Durham's tube was inserted in each test tube. These cultures were cultured in the presence (sub inhibitory concentration) or absence of *A. sativum*, at 37°C for 24-48h. Later the acid formation was tested by addition of few drops of neutral red indicator to the cultures and formation of dark red color is scored as positive. Indole, methyle red and voges proskeur tests were perfomed by secondary cultures cultured in the presence or absence of *A. sativum* in peptone water and MRVP broth (Hi Media) respectively, and incubated at 37°C for 24-48h . Later Kovac's reagent was added to peptone water cultures and formation of cherry red colour was scored as positive. Similarly, methyl red and voges proskeur reagents were added separately to MRVP broth cultures and formation of red color was scored as positive for methyl red and voges proskeur reactions respectively. Citrate utilization was tested by using Simmon's citrate agar slants that were streaked with the primary cultures in the presence and absence of garlic and incubated at 37°C for

24h. The change of medium's green colour to blue is scored as positive. For all these tests, media controls with garlic but without addition of cultures were kept. To study the catalase activity overnight secondary cultures of both control and experimental were treated with 3% hydrogen peroxide taken in a capillary tube and extensive bubble formation in the tube was scored as positive. The oxidase activity was studied by placing the concentrated pellet of secondary broth control and experimental cultures on a piece of filter paper after which one or two drops of 1.0 % Wurster's reagent was placed. If the test culture turns purple it is scored as positive. Urease test was performed by culturing the secondary cultures of these pathogens in the presence and absence of *A. sativum* at 37°C for 18-24h in Christensen's urea broth (Hi Media). Later a few drops of Phenol red indicator was added to the cultures, and development of purple colour is scored as positive. β hemolysis was studied by culturing all pathogens separately as primary broth cultures which were used to prepare the secondary broth cultures in the presence and absence of *A. sativum.* Later a loop full of secondary cultures were streaked on blood agar plate and incubated at 37°C for 18-24h. If there was complete destruction of RBC and any plaque formation on the plate, it was regarded as positive for β-hemolysis. If the RBC were partially destroyed, it was scored as α-hemolysis and when there was no hemolysis, it was scored as γ-hemolysis. Similarly the Deoxy ribonuclease activity was studied by streaking the Tolluidine blue Dnase agar plates (Hi Media) with a loop full of secondary broth cultures prepared in the presence and absence of garlic. These plates were incubated at 37°C for 18-24h. Formation of white colonies with light pink border and degradation of medium was scored as positive. The amylase, proteolytic and phospholipase-C activities were studied by line inoculating each type of bacteria on starch agar, milk agar, and egg yolk agar plates respectively in the presence and absence of *A. sativum* and incubating the plates at 37°C for 24 h. Then starch agar plates were flooded with iodine solution and white zone of hydrolysis around inoculum followed by blue non degraded part was scored as positive. The zone of degradation around inoculum on milk and egg yolk agar were scored as positive.

RESULTS AND DISCUSSION

The results are given in table1. In the present investigation the sub inhibitory concentration of garlic against $1x10^9$cells has induced the morphological alterations such as the highly motile *S. typhimurium*, *S. typhi* and *Y. enterocolitica* at 29°C have become sluggish, and some nonmotile as if they were dead. The gram staining showed shrivelling of the cells and degeneration. The colony morphology of *Y. enterocolitica* appeared less viscous after treatment with *A. sativum* indicating reduction or change in the capsular polysacharide material which is an anti phagocytic agent. The smooth colonies of *S. typhi* and *S. typhimurium* have changed to some what rough colonies after the drug treatment indicating chages in O-polysaccharide chain of LPS of these pathogens. The drug treated colonies of all pathogens showed reduction in colony size.

The biochemical alterations induced by garlic were inhibition of acid formation with various sugars indicating that *A. sativum* has inactivated the enzymes that catalyze these reactions and thereby blocking different fermentation pathways. Similarly the oxidase and urease reactions were negative in *Y. enterocolitica* after *A. sativum* treatment. It indicates that the enzymes oxidase and urease were inactivated by *A. sativum*, respectively. Similarly alterations in methyle red reaction indicates inactivation of ezymes that catalyze mixed acid fermentation reactions, were inactivated there by blocking these pathways. The alteration in β-hemolysis and coagulase reactions followed by garlic treatment indicates inactivation of haemolysin and coagulase enzymes of *Y. enterocolitica* and *S. aureus*, respectively. The Dnase and phospholipase-C activities of *S. aureus* were also inhibited by garlic treatment indicating the inactivation of this enzyme by this herbal medicine. The proteolytic and phospholipase-C enzymes of *Y. enterocolitica* were inactivated by garlic treatment but not the amylase activity which indicates the susceptibility of first two enzymes and resistance of amylase to garlic. These results clearly indicate that *A. sativum* was a potent antimicrobial agent that inactivated a large number of enzymes which are the major virulence factors of these pathogens.

Table 1: The biochemical alterations induced by sub inhibitory concentrations of *A. sativum*

S.No.	*Test*	*Con of A. sativum*	*Y. enterocolitica*	*S. typhimurium*	*S. typhi*	*S. aureus*
1.	Gramstaining	control (-)	gram-ve	gram-ve	gram-ve	gram+ve
		7mg/ml (E)	,,	,,	,,	,,
2.	Motility	control	+ (29°C)	+	+	-
		7mg/ml	- (29°C)	sluggish/-	sluggish/-	-
3.	Lactose	control	A-, G-	A+, G-	A+, G +	A+,G-
		7mg/ml	A-, G-	A-, G-	A-, G-	A-, G-
4.	glucose	control	A+, G-	A+, G+	A+,G+	A+, G-
		7mg/ml	A-, G-	A-, G-	A-, G-	A-, G-
5.	Mannitol	control	A+, G-	A+, G-	A+, G+	A+, G+
		7mg/ml	A-, G-	A-, G-	A-, G-	A-, G-
6.	Catalase	control	+	+	+	+
		7mg/ml	+	+	+	+
7.	Oxidase	control	+	-	-	-
		7mg/ml	-	-	-	-
8.	Urease	control	+	-	-	-
		7mg/ml	-	-	-	-
9.	Hemolysis	control	+	-	-	-
		7mg/ml	-	-	-	-
10.	Indole	control	-	-	-	-
		7mg/ml	-	-	-	-
11.	Methylred	control	+	+	+	+
		7mg/ml	-	-	-	-
12.	Voges proskeur	control	-	-	-	-
		7mg/ml	-	-	-	-
13.	Citrate	control	-	-	-	-
		7mg/ml	-	-	-	-
14.	Coagulase	control	-	-	-	+
		7mg/ml	-	-	-	-
15.	Dnase	control	+	-	-	+
		7mg/ml	-	-	-	-
16.	Amylase	control	+	-	-	-
		7mg/ml	+	-	-	-
17.	Proteolysis	control	+	-	-	-
		7mg/ml	-	-	-	-
18.	lecithinase	control	+	-	-	+
		7mg/ml	-	-	-	-

A = acid; G = gas ; E = experimental; + = positive; - = negative; control = no garlic

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KEY WORDS Antimicrobial Activity. *A. sativum*. Biochemical Alterations. Microbial Pathogens.

ABSTRACT The morphological and biochemical alterations induced by sub inhibitory concentrations of *Allium sativum* ($7mgml^{-1}$) on three gram negative bacteria viz. *Yersinia enterocolitica*, *Salmonella typhimurium* and *Salmonella typhi*, and a gram positive bacterium such as *Staphylococcus aureus* ($1x10^{9}$cells ml^{-1} of each culture) were studied. The size of the colony was reduced on solid media, less viscous capsular colonies appeared (*Y. enterocolitica*), the highly motile organisms have become sluggish/ non motile/ dead (*S. typhi*, *S. typhimurium* at 37°C and *Y. enterocolitica* at 29°C) as determined by hanging drop method. There were alterations in the fermentation reactions of various sugars such as, glucose, lactose and mannitol by these microbes, indicating that *A. sativum* has inactivated several enzymes involved in different fermentation pathways thereby blocking these path ways. The oxidase, β-hemolytic, Proteolytic,Dnase and lecithinase enzymes have been inactivated but not the amylase activity in *Y. enterocolitica* following treatment with *A. sativum*. There was alteration in methyl red reactions followed by treatment with *A. sativum* in these pathogens indicating the inactivation of enzymes involved in mixed acid fermentation reactions of these pathogens. The enzymes coagulase, Dnase and

phospholipase-c were inactivated in *S. aureus*. These results clearly indicate that *A. sativum* is a potent antimicrobial agent which inactivated several enzymes at sub inhibitory concentration, eventually leading to the death of these pathogens.

REFERENCES

Cappuccino, J.G., and Sherman, N.: *Microbiology a Laboratory Manual*. 4th Edition. an inprint of Addison Wesley Longmann, Inc (1999)

Elizabeth, K. M.: Antimicrobial activity of *Rauwolfia serpentina. J. Hum. Ecol.*, **12 (3):** 875-877 (2001).

Elizabeth, K. M.: Antimicrobial activity of *Alhin sativum* as some human pathogens. *Indian J. Microbial.*, **48** (2001).

Farnsworth, N. R. Screening plants for new medicines. In: *Biodiversity*, E.O.: Wilson. (Ed.). National Academy press: Washington, D.C., 83-97 (1988)

Haraguchi,H., Kataoka,S., Okamoto,S.,Hanafi,M., Shibata, K.: Antimicrobial triterpenes from *Ilex integra* and mechanism of antifungal action. *Phytother. Res.*, **13:** 151-156 (1999).

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