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Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish

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ABSTRACT

The antibacterial effect of aqueous garlic and cinnamon extract at five different temperatures (40^O C, 60^O C, 80^O C, 100^O C, 120^O C) against five multidrug resistant bacterial isolates (2 gram negative and 3 gram positive), including Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, E. Coli and Proteus mirabilis were studied by well diffusion method. The maximum antibacterial effect of aqueous garlic and cinnamon extract of different temperature obtained in Enterococcus faecalis and E. Coli at 60^O C (1.041) and in Enterococcus faecalis at 60^O C (0.87) respectively. This antibacterial property was applied on preservation of fish. A time dependent antibacterial study was done using the gum acacia coating with garlic and cinnamon paste. It is observed that the microbial load present on the fish was totally reduced on second day. In short, the aqueous garlic and cinnamon extract and pastes show a wide range of antibacterial activity at 40^O C to 60^O C and satisfy all the criteria for antibacterial agent as compared to antibiotic Gentamicin. These results suggests that garlic and cinnamon can be used as food preservative and thus the use of other chemical preservatives can be minimized, which would be beneficial for environment and consumer health; or a plastic for food preservation can be invented using the antibacterial activity of garlic and cinnamon, the inner wall of the plastic coated with garlic and cinnamon paste.

Keywords: Aqueous garlic extract, Aqueous cinnamon extract, Gentamicin, activity index, fish and coating.

INTRODUCTION

There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives [1,20]. Extract from many plants used as flavouring and seasoning agents in food and beverages have been used therapeutically for centuries [2,21]. However, there is a little data on antimicrobial activities and most medicinal plants [3]. The antimicrobial activities of garlic, cinnamon, onion and cloves have been studied since the end of the last century and the active compounds in these herbs were determined [4]. Cinnamon contains 0.5 to 1.0% volatile oil composed mainly of cinnamyldehyde (50.5%), eugenol (4.7%), cinnamic acid, methoxycinnamaldehyde (MOCA) and cinnamyl acetate (8.7%) [5]. The active compound present in garlic is the allicin, allyl alcohol which is a thiosuffinate compound reported for its antimicrobial activity [6].

Historically, garlic and cinnamon has been used for centuries worldwide by various societies to combat infectious diseases. Cinnamon is reputed as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system [7]. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices.

Garlic (*Allium sativum L.)* exhibit a broad antibiotic activity against both gram negative and gram positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Rlebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Helicobacter*, *Pylori* [8] and on diarrheagenic organisms [9]. Cinnamon has been reported to inhibit the growth of several antibiotic resistant strains of bacteria [10] and antibacterial activity of commercial and wild – cinnamon species has been seen[11]. Cinnamon in concentration as low as 0.02% inhibited mold growth and aflatoxin production in yeast extract sucrose broth [12]. The raw juice of garlic was effective against many common

pathogenic bacteria[22], against the strains that have become resistant to antibiotics and even toxin production by some pathogenic strains prevented by garlic [13].Cinnamon, which is high in antioxidant and antimicrobial activity [14] by the mechanism of decreasing the DNA binding activity of the quorum sensing response regulator Lux R[15]. Allicin, the active ingredient of garlic acts by partial inhibition of DNA and protein synthesis and also total inhibition of RNA synthesis as a primary target. Similar to garlic ampicillin inhibit cell wall synthesis by inhibiting transpeptidation enzyme involved in cross linking of polysaccharide chain of bacterial cell wall and also activate cyclic enzyme [16].

Garlic and cinnamon used for food preservation because of their bacteriocin based strategies [17] and garlic and cinnamon are used as natural preservatives in poultry and meat products [18]. Also cinnamon, essential oil is used in fish preservation [19].

MATERIALS AND METHODS

Microorganisms used: A total of 2 gram negative and 3 gram positive organisms were used in the study. The isolates *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *E. Coli* and *Proteus mirabilis* were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Media Used: The media used in the present investigation was Nutrient Agar media, which was obtained from Hi – Media Laboratories Ltd., Mumbai, India.

Antibiotic Gentamicin used: The antibiotic standard used in this investigation was Gentamicin G30 susceptibility test discs (30 mg per disc) which were obtained from Hi – Media Laboratories Ltd., Mumbai, India.

Garlic and Cinnamon aqueous stock preparations: Fresh garlic (*Allium sativum L.*) bulbs and cinnamon (*Cinnamonum zeylanicum*) bark were purchased from a retail food store (Vellore, India). Then, the garlic bulbs were peeled. 15 gram of surface sterilised garlic bulbs and cinnamon bark were taken separately in 5 different sterilized petriplate and were incubated at several temperatures (40^O C, 60^O C, 80^O C, 100^O C and 120^O C) for 20 minutes. Now these samples were separately homogenised aseptically in 75 ml of sterile distilled water in surface sterilized electric grinder and then filtered in conical flasks. The filtrates were used as aqueous stock for antibacterial activity and were stored in the refrigerator.

Re – culturing of micro organisms used or Enrichment of culture: The bacterial cultures were maintained in Peptone Saline Water.

Comparison of antibacterial activity of Garlic and Cinnamon at different temperature using Agar well diffusion assay: 100 µl of each bacterial strain was used to make lawn culture on sterile Nutrient Agar Plates. 6 mm wells (5 numbers in a plate) were made using cork borer. To each well 100 µl of each Garlic and cinnamon stocks of different temperature were added respectively. 100 µl of aqueous Garlic and cinnamon extract contained 8.5 mg of total dry matter and 1.65 mg respectively .There should be sufficient distance between the wells to avoid overlapping of zone of inhibition. The plates were incubated in an upright position at 37^O C for 24 hours. The diameter of zone of inhibition was measured in millimetre and the results were recorded. The inhibition zones with diameter less than 8 mm were considered as having antibacterial activity. All the media used in the present investigation were obtained from Hi – media laboratories Ltd., Mumbai, India.

Antibiotic sensitivity testing: The test microorganisms were also tested for their sensitivity against the antibiotic (Gentamicin 50 mg). Using sterile cotton swabs, the enriched cultures were aseptically swabbed on the surface of sterile nutrient agar plates. Using a ethanol dipped and flamed forceps, the antibiotic antimicrobial disc was aseptically placed over the agar plates sufficiently separated from the wells formed to avoid overlapping of inhibition zone. The plates were incubated at 37 C for 24 hours and the diameter of inhibition zones were measured in mm. All the media used in the present investigation were obtained from Hi – media laboratories Ltd., Mumbai, India.

Measuring Activity Index: Following formula was used to measure Activity Index, Activity Index = (Zone of inhibition of extract/Zone of inhibition of antibiotic). Zone of inhibition of stocks at different temperature was measured and similarly zone of inhibition of Antibiotic (Gentamicin) was measured.

Garlic and Cinnamon Paste preparation: The peeled garlic bulb and cinnamon bark were weighed 15g, cleaned garlic and cinnamon was taken and surface sterilized. Garlic and cinnamon were separately homogenised aseptically

in 4ml of sterilized distilled water in surface sterilized electrical grinder. The paste of garlic and cinnamon were taken in two separate sterilized beakers.

Formulation of coating with and without extracts 6g of fish: Acacia gum (2 gram), sterilized distilled water (1 ml) were taken in two sterilized beakers. Then it is dielectrically heated in microwave oven for 10 seconds. 0.33 gram of each extracts was added in one of the beakers.

Antibacterial activity of coating with extract and without extract on fish: Pieces of fish (6g) were coated with gum acacia solution containing paste and without paste respectively. Control was kept without coating. Then they were incubated for 45 minutes at 55^{O} C so that the coating sticks properly over the surface of the fish. Now these uncoated and coated samples were transferred into three separate zip lock plastic pouches using forceps. After each 24 hour interval streaking was done to see the reduction in microbial load, until there was negligible growth.

RESULTS AND DISCUSSION

Activity Index of Garlic and Cinnamon at Different Temperature: The zone of inhibition for Gentamicin was measured (Table 1) and average activity index of garlic and cinnamon for 2 gram negative and 3 gram positive bacteria was calculated at different temperature (Table 2).

For *Proteus mirabilis*, garlic and cinnamon has maximum average activity index at 60^{O} C which is 0.706 and 0.676 respectively and average activity index of garlic and cinnamon is more than 0.5 at 60^{O} C to 100^{O} C and 40^{O} C to 60^{O} C respectively.

Table1: Zone of Inhibition for Gentamicin

Name of Organism	Zone of Inhibition for Gentamicin
Proteus mirabilis	2.5
Enterococcus faecalis	2.4
Bacillus cereus	2.8
E. Coli	2.1
Staphylococcus aureus	4.2

Table 2: Average Activity index of garlic and cinnamon for 2 gram negative and 3 gram positive bacteria was calculated at different temperature

Name of Organism	Extract	40^{O} C		60^{O} C		80^{O} C		100^{O} C		120^{O} C	
		Zone of Inhibition of Extract (Cm)	Average Activity Index	Zone of Inhibition of Extract (Cm)	Average Activity Index	Zone of Inhibition of Extract (Cm)	Average Activity Index	Zone of Inhibition of Extract (Cm)	Average Activity Index	Zone of Inhibition of Extract (Cm)	Average Activity Index
Proteus mirabilis	**Garlic**	1.4	0.613	1.5	0.706	1.4	0.593	1.3	0.563	1.1	0.476
	Cinnamon	1.5	0.640	1.6	0.676	1.2	0.513	1.1	0.480	1.1	0.466
Enterococcus faecalis	**Garlic**	2.0	0.870	2.5	**1.077**	1.8	0.833	1.8	0.783	1.7	0.740
	Cinnamon	1.9	0.833	2.1	**0.923**	1.4	0.626	1.2	0.573	1.2	0.560
Bacillus cereus	**Garlic**	1.2	0.480	2.0	0.773	1.5	0.596	1.4	0.563	1.4	0.526
	Cinnamon	1.2	0.470	1.2	0.486	1.6	0.626	1.6	0.600	1.6	0.576
E. Coli	**Garlic**	1.2	0.673	2.2	**1.074**	1.4	0.706	1.4	0.683	1.3	0.640
	Cinnamon	1.1	0.583	1.5	0.760	1.4	0.693	1.3	0.666	1.2	0.616
Staphylococcus aureus	**Garlic**	1.5	0.403	2.2	0.600	1.5	0.382	1.4	0,366	1.2	0.323
	Cinnamon	1.1	0.303	1.8	0.463	1.1	0.326	1.3	0.302	1.2	0.283

For *Enterococcus faecalis*, garlic and cinnamon has maximum average activity index at 60^{O} C which is 1.077 and 0.923 respectively and average activity index of garlic and cinnamon is more than 0.5 at 40^{O} C to 120^{O} C for both.

For *Bacillus cereus*, garlic and cinnamon has maximum average activity index at 60^{O} C and 80^{O} which is 0.773 and 0.626 respectively and average activity index of garlic and cinnamon is more than 0.5 at 40^{O} C to 120^{O} C for each.

For *E. Coli*, garlic and cinnamon has maximum average activity index at 60^{O} C which is 1.074 and 0.760 respectively and average activity index of garlic and cinnamon is more than 0.5 at 40^{O} C to 120^{O} C for each.

For *Staphylococcus aureus*, garlic and cinnamon has maximum activity index at 60^{O} C which is 0.600 and 0.463 respectively and only garlic shows activity index more than 0.5 at 60^{O} C only.

In most of the bacteria (*Bacillus cereus*, *Euterococeus faecalis*, *E. Coli*) both garlic and cinnamon shows antibiotic activity till 120^{O} C, so garlic and cinnamon can be used as a food preservative for fried and deep fried food.

Garlic and cinnamon shows maximum average activity index between 60^{O} C to 80^{O} C.

Zone of Inhibition for Gentamicin Sensitivity Test

From the graphs (Figure 1) it can be said that generally there is a slight decrement in activity index of garlic and cinnamon as the temperature increases from 60^{O} C.

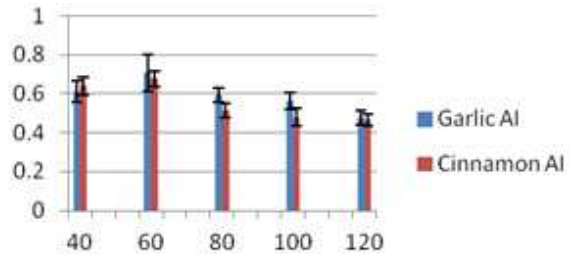

Figure 1.1 *Proteus mirabilis*

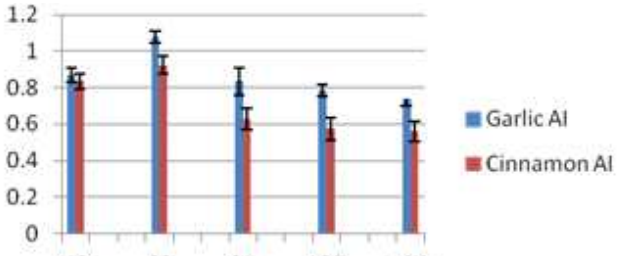

Figure 1.2 *Euterococeus falealis*

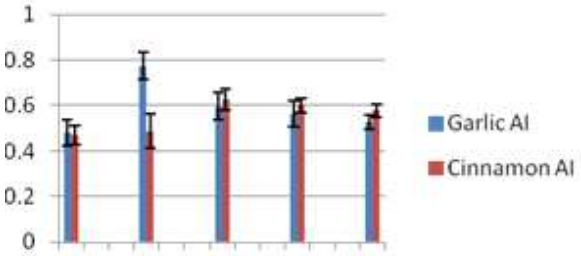

Figure 1.3 *Bacillus cereus*

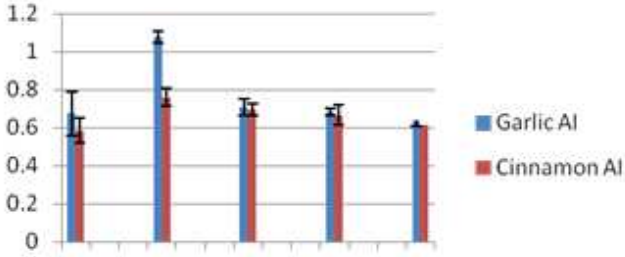

Figure 1.4 *E.Coli*

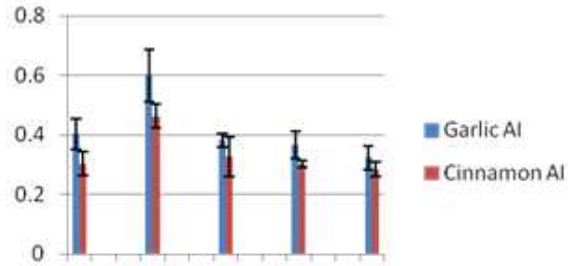

Figure 1.5 *Staphylococcus aureus*

Figure 1: Graphical representation for activity index of garlic and cinnamon at different temperatures on different Bacteria (X axis temperature in ^{0}C and Y axis is average activity index)

Antibacterial Activity of Garlic and Cinnamon Paste on Fish

For coating when 2 gram of gum acacia1 ml sterilized distilled water and 0.33 gram of each extract was used then antibacterial activity of coating with extract seen on 5^{th} day; but when 2 gram of gum acacia, 1 ml of sterilized distilled water and 0.5 gram of each extract was used then the antibacterial activity of coating with extract seen on 2^{nd} day.

There was no antibacterial activity on sample coated without extract and on sample without coating. So, it can be said that garlic and cinnamon paste can be used in fish preservation in more pure form.

Figure 2.1: Zone of Inhibition by Cinnamon on *Bacillus cereus* at Different Temperature

Figure 2.2: Zone of Inhibition by Garlic on *Bacillus cereus* at Different Temperature

Figure 2.3: Zone of Inhibition by Cinnamon on *E. Coli* at Different Temperature

Figure 2.4: Zone of Inhibition by Garlic on *E. Coli* at Different Temperature

Figure 2.5: Zone of Inhibition by Cinnamon on *Eutevococeus falealis* at Different Temperature

Figure 2.6: Zone of Inhibition by Garlic on *Eutevococeus falealis* at Different Temperature

Figure 2.7: Zone of Inhibition by Cinnamon on *Proteus mirabilis* at Different Temperature

Figure 2.8: Zone of Inhibition by Garlic on *Proteus mirabilis* at Different Temperature

Figure 2.9: Zone of Inhibition by Cinnamon on *Staphylococcus aureus* at Different Temperature

Figure 2.10: Zone of Inhibition by Garlic on *Staphylococcus aureus* at Different Temperature

Figure 2: Shows the Antibacterial Activity of Garlic and Cinnamon at Different Temperature by Well Diffusion Method

Figure 3.1.1: Control and sample coated with extract in zip pouch

Figure 3.1.2: Sample coated with extract and sample coated without extract in zip pouch

Figure 3.1.3: Control, Sample coated with extract and sample coated without extract on 0^{th} hour

Figure 3.1.4: Control, Sample coated with extract and sample coated without extract on 1^{st} day

Figure 3.1.5: Sample coated without extract and sample coated with extract on 2nd day

Figure 3.1.6: Control, Sample coated without extract on 2nd day

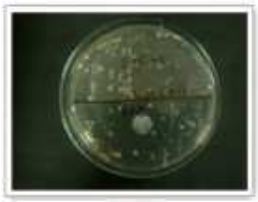

Figure 3.1.7: Sample coated without extract and sample coated with extract on 3rd day

Figure 3.1.8: Control, Sample coated without extract on 3rd day

Figure 3.1.8: Sample coated with extract and sample coated without extract on 4th day

Figure 3.1.9: Control, Sample coated without extract on 4th day

Figure 3.1.10: Sample coated with extract and sample coated without extract on 5th day

Figure 3.1.11: Control, Sample coated without extract on 5th day

Figure 3: shows the anti bacterial activity of garlic coating with extract, without extract on fish and control which is without coating for Trial 1 and Trail 2

CONCLUSION

The activity index of garlic and cinnamon for different temperature was calculated after antibacterial testing of garlic and cinnamon at different temperature using agar wall diffusion method and antibacterial sensitivity testing with Gentamicin. Here activity index of garlic and cinnamon more than 0.5 is observed for approximate 60^{O} C to 120^{O} C (except *Staphylococcus aureus* and *Proteus mirabilis*). Garlic and cinnamon extracts were used without any purification and then also they are showing better activity index, so this result encouraged to the antibacterial activity of coating with and without extract on fish. Maximum antibacterial activity of coating with extract was on 5^{th} day and 2^{nd} day when different amount of gum acacia and extracts of cinnamon and garlic was used. These results gives the good evidence that garlic and cinnamon can be used as a natural food preservations for fish at room temperature, fried fish, deep fried fish since the activity index of the garlic and cinnamon is more than 0.5 till 120^{O} C. garlic and cinnamon extracts can be the best replacement of chemical preservatives when their extracts are used after purification and thus the use of other harmful chemical preservatives can be minimized which will be beneficial for health. Based on the above result, a new type of plastic can be invented by applying engineering technologies; the inner wall of plastic contains the dried coating of garlic and cinnamon paste and thus food ingredients can be stored in it, because the entry of bacteria and other microbes is minimized by garlic and cinnamon coating in the plastic.

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