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Studies on Antimicrobial Activity of Spices and Effect of Temperature and Ph on Its Antimicrobial Properties

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Abstract: *Spices are reported to be antibacterial and have medicinal values. Spices such as garlic (Allium sativum), ginger (Zingiber officinale), clove (Eugenia caryophyllata), cinnamon (Cinnamomum zeylanicum) posses antimicrobial effect and has been used traditionally. In this study we studied the antibacterial activity of spices, effect of temperature and pH on its antibacterial effect. It was tested against four test microorganisms Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi by aqueous extracts of spices using agar diffusion method. On observation, it was found that all the spices showed inhibitory effect and garlic showed maximum inhibition on Escherichia coli and Bacillus subtilis. On treating the extracts of garlic and ginger at both room temperature (26°C) and at higher temperature (100°C) it was seen that garlic has significantly greater zone of inhibition than ginger. Therefore higher temperature reduces the antibacterial effect of both extract, but garlic retains its antibacterial activity. On treating the garlic extract with different pH, it was seen that the antibacterial effect was decreasing on increasing with pH but the antibacterial property was never lost. Therefore garlic can be used as an effective source for food preservation and also as a natural herbal antibiotic with or without boiling.*

Keywords: *Antibacterial effect, garlic, spices, pH, Temperature.*

I. Introduction

The use of several chemicals in food and also use of several antibiotic medicines has made bacteria to develop resistance in their population. Spices have got excellent antibacterial properties, in many countries people use these along with boiled food preparations which actually reduce its antibacterial properties [1]. Spices such as garlic, ginger, clove and cinnamon has been used traditionally for both culinary and medicinal purposes. In India garlic has been used to cure wound infection and food spoilage [2]. Little information is available on the preservative and antimicrobial role of spices and their oils and the role of various components of essential oils in the preservation of spoil of food [3]. To prevent food spoilage, chemical preservatives are used to prevent the growth of food spoiling microbes in the food industry [4]. There has been constant increase in search of alternate and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives [5, 6]. Also there has been increase of consumers about foods free or with lower level of chemical preservative because these could be toxic for humans [7]. Therefore, Plant materials with antibacterial properties have a possible application in food preservation. Several studies have proved that has garlic has antimicrobial effects [2, 8-13]. Garlic extract exhibits broad spectrum antimicrobial activity against many genera of bacteria and fungi at room temperature [14]. The present study deals with the study of antibacterial activity of various spices, how temperature affects the antibacterial properties of spices and stability of antibacterial activity of garlic at different pH therefore to understand its potential as food preservation and also as a natural herbal antibiotic.

II. Materials And Method

It was carried out with common spices like garlic (Allium sativum), ginger (Zingiber officinale), clove (Eugenia caryophyllata) and cinnamon (Cinnamomum zeylanicum), which were obtained from local market (Sion, India). Then these spices were grounded in sterile mortar and pestle with distilled water and then filtered with muslin cloth. Therefore these filtrates were used as aqueous stock for antibacterial activity.

Agar well diffusion assay for antibacterial activity of different spices

The agar well diffusion method was carried out to study the antibacterial activity of spices. Nutrient agar medium was prepared to grow the test organisms of two Gram positive and two Gram negative bacteria such as Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi. The test bacterial culture 0.1 ml was inoculated in a Petri plate containing nutrient agar and spread evenly with sterile metal spreader under sterilized conditions. A hole of 9 mm diameter was made in a nutrient agar plate containing test organisms by using sterilized cork borer and 0.1 ml of aqueous extract of garlic, ginger, clove and cinnamon were poured in those well using a micropipettes. Then these inoculated nutrient agar plates were incubated at 37°C for 18 hrs in the incubator. After incubation the zone of inhibition were observed from the size of diameter and measured in mm.

Antibiotic Streptomycin

The streptomycin susceptibility test discs are used as an antibiotic standard in this study.

Effect of temperature on antibacterial activity of garlic and ginger

The effect of temperature on its antibacterial activity is determined by taking aqueous extracts of garlic and ginger in sterile test tubes and kept at room temperature (26°C) and other boiled at higher temperature (100°C). Then the antibacterial activity was tested by agar well diffusion method.

Effect of pH on antibacterial activity of garlic

The effect of pH on stability of antibacterial activity of garlic was determined by adjusting the pH (5.8, 6, 7, 8, and 9) at room temperature with 1N NaOH and allowed it to stand for 2 hours. Then antibacterial activity was tested by again agar well diffusion method.

III. Results And Discussion

Antibacterial activity of spices

The zone of inhibition for streptomycin antibiotic was measured (Table 1). The antibacterial activity of garlic, ginger, clove and cinnamon against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi was observed (Table 2). All the spices showed zone of inhibition in which garlic showed maximum inhibitory effect with Escherichia coli (31 mm) and Bacillus subtilis (29 mm). Ginger and clove was found to inhibit Bacillus subtilis at moderate level. On contrast cinnamon inhibited Bacillus subtilis and Staphylococcus aureus to a greater extent in comparison with ginger and clove. According to (Arora and Kaur) [2] garlic inhibited the growth of many bacteria with Sh. Flexneri is more susceptible and E. aerogenes the least and clove inhibited the growth of Sh. Flexneri to a moderate extent. In this study we found that all the spices inhibited the growth of bacteria.

Table 1: Zone of inhibition of streptomycin.

Bacteria	**Zone of inhibition (mm)**
Bacillus subtilis	35
Staphylococcus aureus	33
Escherichia coli	29
Salmonella typhi	31

Table 2: Antibacterial activity of garlic, ginger, clove and cinnamon

Bacteria	**Zone of inhibition (mm)**			
	Garlic	**Ginger**	**Clove**	**Cinnamon**
B. subtilis	**29**	**15**	**13**	**19**
S. aureus	**26**	**16**	**17**	**16**
E. coli	**31**	**12**	**14**	**17**
S. typhi	**25**	**14**	**12**	**13**

Effect of temperature on antibacterial effect of garlic and ginger

Extracts of garlic and ginger were kept at normal room temperature (26°C) and at higher temperature (100°C).It was observed that there is a decrease in the antibacterial activity of garlic and ginger at higher temperature (100°C) (Table 3), but the antibacterial activity of garlic sustained at higher temperature, on the contrary ginger showed negligible antibacterial activity on all the bacteria. Previous studies have showed that garlic retains its antibacterial effect even at higher temperature [1]. Our results are in accord with Ranjan et al where they showed that garlic retains it antimicrobial activity up to 120^0C.

Table 3: Antibacterial effect of garlic and ginger at different temperatures

Bacteria	**Zone of inhibition (mm)**			
	Garlic		**Ginger**	
	26°C	**100°C**	**26°C**	**100°C**
B. subtilis	29	20	15	0
S. aureus	26	18	16	0
E. coli	31	15	12	0
S. typhi	25	14	14	0

Effect of pH on antibacterial activity of garlic

Effect of pH on stability of antibacterial activity of garlic is tested by adjusting the aqueous extract pH such as 6, 7, 8 and 9. On observation it was found that there is a decrease in antibacterial activity of garlic as the pH increases. The antibacterial activity was greater in the normal room temperature (5.8). The findings of our

test are similar with (Tynecka et al) [16] and (Srinivasan Durairaj) [17] in which the antimicrobial activity of Allium ursinum juice and garlic extract was decrease with increase in pH. Although there is a decrease in the antibacterial activity, garlic maintained its antibacterial property up to some extend of varying pH (Table 4).

Table 4: Antibacterial activity of garlic at different pH

Bacteria	Zone of inhibition (mm)				
			pH		
	5.8	**6**	**7**	**8**	**9**
B. subtilis	29	25	20	16	14
S. aureus	26	22	15	13	0
E. coli	31	26	19	14	12
S.typhi	25	19	13	12	0

Fig 1: Graphical representation of zone of inhibition of garlic, ginger, clove and cinnamon against different bacteria

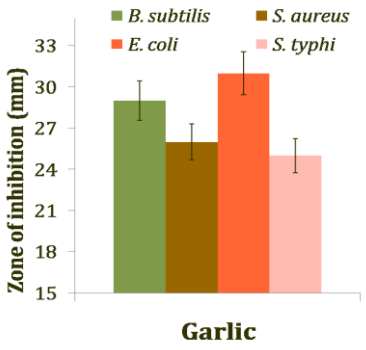


Fig 1.1: Zone of inhibition of garlic

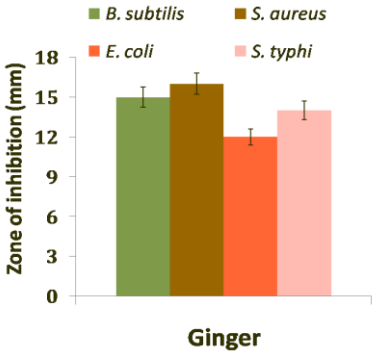


Fig 1.2: Zone of inhibition of ginger

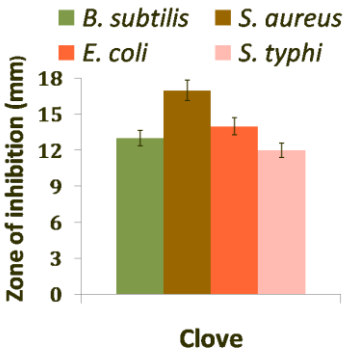


Fig 1.3: Zone of inhibition of clove

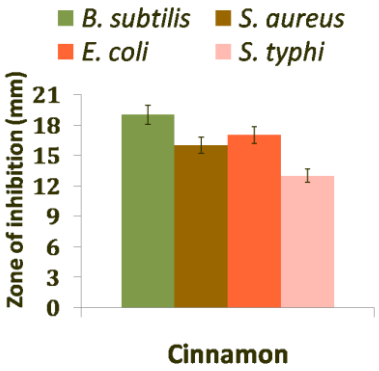
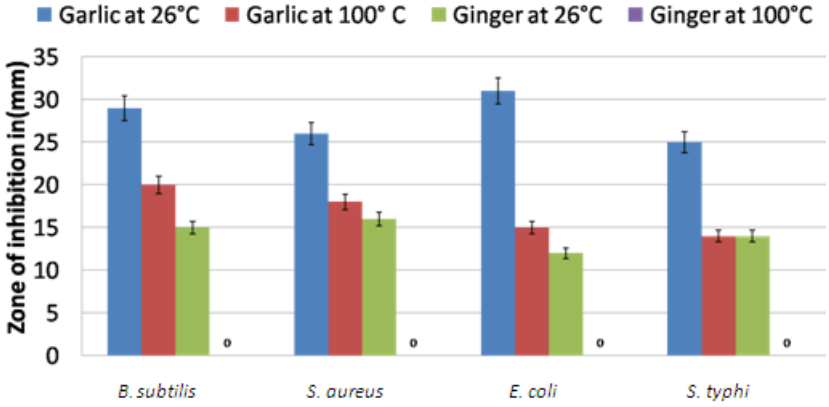


Fig 1.4: Zone of inhibition of cinnamon

Fig 2: Graphical representation of zone of inhibition of garlic and ginger at different temperature.



IV. Conclusion

The antibacterial activity of different spices was calculated by agar well diffusion method. The results showed that all the spices showed inhibitory effect. Garlic showed maximum inhibitory effect among different spices. The antibacterial sensitivity testing was done with standard streptomycin. The antibacterial effect at higher temperature was done with garlic and ginger and it was seen that there is a decrease in the antibacterial activity at higher temperature (100°C). The decrease in the antibacterial activity is may be due physical or chemical changes that occur during heating the extracts. People use these spices either directly or with boiled food preparations in which it destroys their antibacterial properties. In this study we saw that how temperature affects the antibacterial properties of spices. On treating the garlic extract at various pH it was observed that there is a decrease in the antibacterial activity with increase in pH. Although the antibacterial effect of garlic decreases with increase in temperature and pH the antibacterial activity was sustained. Therefore garlic retained its antibacterial effect even at different pH and also at higher temperature. Numerous studies have showed that allicin present in garlic has an antibacterial effect. Allicin in its pure form, exhibit antibacterial activity against a wide range of Gram negative and Gram positive bacteria including multidrug strains of E. coli[18]. The results supports that garlic has more potential as an antibacterial source which can be used as effective food preservation and also as an antibiotic. The findings of our studies elaborate the antibacterial properties of different spices and effective antibacterial properties of garlic under various conditions. Therefore garlic can be used as an effective antibacterial source against many bacterial diseases and also used as a natural food preservative as they exhibit effective antibacterial properties under various conditions.

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