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ANTIBACTERIAL EFFECT OF GARLIC AND GINGER EXTRACTS ON *Escherichia coli* and *Listeria monocytogenes*

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Abstract

Investigations were carried out to study the inhibitory effect of ginger (Zingiber officinale) and garlic (Allium sativum) extracts at 0.5, 1.0, 2.0, 7.0 and 10.0 per cent level in vitro on Escherichia coli and Listeria monocytogenes by spectrophotometer method. Both the spices exhibited bacteriostatic effect against both the test organisms. The inhibitory effect increased with the increase in the concentration of extracts. The garlic extract was more effective against E. coli than L. monocytogenes. Ginger extract was effective in restricting the growth of E. coli at 0.5, 1.0 and 2.0 per cent level. The minimum inhibitory effect of ginger extract was found to be 8.7 and 8.5 for E. coli and L. monocytogenes respectively, whereas in case of garlic extract it was 9.1 and 8.0, respectively. It was concluded that both the spices possess a good potential to act as natural preservative.

I. INTRODUCTION

Listeria monocytogenes and *Escherichia coli* have been isolated from various types of foods and associated with food borne infection in man. The presence of these organisms and their growth and multiplication in food do not produce much appreciable changes in foods but leads to food borne infection. In order to prevent the growth of bacterial pathogens in food, various preservative techniques have been used[1,2]. Consumer concerns on the safety of foods containing synthetic chemicals as preservatives have resulted in a growing need for use of natural antibacterial compounds, having a characteristic flavour, antioxidant and antimicrobial activity[3,4]. Currently, various natural compounds like spices are preferred and used as food preservatives [3,5]. Kerala, a south Indian state in tropical asia is a well endowed state with numerous varieties of spices. Spices form an integral part of the traditional cuisines in the everyday diet of the common man here[6,7,8]. With globalisation and increased recreational travel, intercontinental modification in the local cuisines has resulted in vast changes in traditional cooking methods. The anti-microbial properties of various natural spices against emerging pathogens have to be taken advantage of, with the changing lifestyle.

Bactericidal or bacteriostatic activity of essential oils in spices against various pathogens including *Escherichia coli* O157:H7 and *Listeria monocytogenes* have been reported [9,10]. The present study was undertaken to assess the direct effect of two of the most commonly used spices, ginger and garlic extracts on *Listeria monocytogenes* and *Escherichia coli.* The minimum inhibitory concentrations were evaluated to enable the use of these for microbial control and as food preservative specifically against these two organisms.

II. MATERIALS AND METHODS

2.1 Preparation of Extracts

In order to prepare fresh garlic (Allium sativum) extract, fresh garlic was purchased from the local market, deskinned and was washed with 0.2% mercuric chloride solution for 3-4 times and then washed 5-6 times in sterile distilled water. About 100g of this washed garlic was mashed in sterile mortar and pestle and sieved through sterile muslin cloth to obtain the extract. From this 0.5, 1.0, 2.0, 4.0, 7.0 and 10.0 per cent of the extract was prepared. Fresh extract was prepared at each time of the experiment.

The fresh extract of ginger (Zingiber officinale) was prepared as described for the preparation of garlic.

2.2. Bacterial Cultures

The standard culture of *Listeria monocytogenes* (MTCC-657) was obtained from the Institute of Microbial Technology, Chandigarh and *Escherichia coli* (V517) was obtained from the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. These isolates were maintained in Nutrient agar media.

In order to test each isolate, it was subcultured in Brain Heart Infusion broth (Hi-Media, Mumbai) and was incubated at 37°C for overnight. The serial dilution of the culture was made in sterile normal saline solution (NSS). All overnight cultures were standardized using the MacFarland turbidity standard using sterile normal saline. The count of the serially diluted samples was also estimated by pour plate method [11]. The count per ml of the samples was estimated by multiplying the mean count of each dilution with the dilution factor. The dilution with a count of 10^5 organisms per ml was selected for the estimation of antibacterial effect of ginger and garlic extract [12].

2.3. Estimation of Inhibitory Effect

The sterile brain heart infusion broth (9ml) each was transferred into fourteen tubes, with 1ml each of the inoculums containing 6.6 x 10^5 of *Listeria monocytogenes*. One ml of each concentration of the garlic extract were added in duplicate tubes containing the inoculums. Two of the tubes were kept as control without the addition of the extract. The optical density (OD) of both the experimental and control tubes were determined by spectrophotometer at 620 nm [13]. Then the control and experimental tubes were incubated at 37°C for 24 hours. At the end of the incubation period, the OD of each tube was determined as before. The experiment with the extract at the above concentration was repeated six times. The difference between the final and initial readings was calculated and interpreted as the growth of bacteria whereas the comparison of the final readings with the control reading depicted the inhibitory effect of the spices on bacterial cultures.

Inhibitory effect on *Escherichia coli* was determined as in case of *Listeria monocytogenes* but the initial inoculum contained 7.2 x 10^5 cfu/ml.

Similar procedure was followed to estimate the inhibitory effect of ginger extract of 0.5, 1.0, 2.0, 4.0, 7.0 and 10.0 per cent concentration on *Escherichia coli* and *Listeria monocytogenes*.

Minimum inhibitory concentration (MIC) of the extracts of ginger was determined by plotting a graph using change in OD in the Y-axis against the concentration of extracts in the X-axis [13]. From the point of intersection, on the curve, a perpendicular was drawn to the X-axis represented the concentration of extract, which inhibited 80% of the test organisms and was designated as MIC.

Data was subjected to statistical analysis using ANOVA and paired t test [14].

III. RESULTS AND DISCUSSION

The antibacterial effect of ginger (Zingiber officinale) and garlic (Allium sativum) extract on *Escherichia coli* and *Listeria monocytogenes* was determined invitro by spectrophotometer method.

The effect of these spice extracts at varying concentrations on *E.coli* is shown in Table I. From the table, it may be observed that the inhibitory effect of garlic and ginger extract increases with the increase in the concentration of the extracts. Similar observation was also reported by Ural *et al*.[15]. The inhibitory effect of ginger extract was much higher at all concentrations as compared to garlic extract on the organism. The antibacterial effect of ginger extract at 0.5, 1.0 and 2.0% level was significantly ($p<0.05$) higher than that of garlic extract on *E. coli*. The observation of the study also indicate that the inhibitory effect of Allicin (active principle of garlic) on Gram positive bacteria was more as compared to Gram negative bacteria. Both the extracts were found to produce bacteriostatic effect at all concentrations.

The MIC of the garlic and ginger extracts on *E. coli* is shown in Fig. 1. The MIC required for the inhibition of 80% growth of the organism was 9.1 and 8.7 per cent of garlic and ginger extract, respectively. In a study [16], it was observed that garlic at different concentrations (25%, 50%, 75% and 100%) had good antibacterial action against a number of *E. coli* serogroups, whereas ginger had only moderate antibacterial action. Aqueous extracts of ginger had no significant antibacterial effect on *E. coli* [17]. The per cent of garlic extract required to produce 80% inhibition of the organism observed in the study was much higher than that recorded in another study [13], where it was observed that 4% of the extract produced the above effect. This may be attributed to the difference in the strain of *E. coli* used in both studies and also the difference in the variety of garlic used in the above studies. While some workers [18, 19] observed that the antimicrobial activity of clove on *E. coli* was more than other spices like ginger, garlic, mustard, amla, aloe vera and saffron, another worker [20] reported that turmeric had more antibacterial effect on *E. coli* than ginger, cinnamon, clove and asafoetida.

The inhibitory effect of these spice extracts at varying concentration on *Listeria monocytogenes* is shown in Table-2. Both ginger and garlic extracts have bacteriostatic effect on the organism. The inhibitory effect of garlic extract at 0.5 per cent level was significant ($P\leq0.5$) as compared to the effect of ginger. Increase in concentration of extracts inhibit the growth of bacteria, which is indicated by the reduction in OD value. The above observation of the study was in accordance with the findings of some workers [13, 21, 22], while in another study [3], it was observed that the essential oil of ginger extracted by hydrodistillation had the highest efficiency against *L. monocytogenes*, with a minimum concentration of inhibition being 6.25 µg/ml.

The MIC of the extracts as shown in Fig.2, was found to be 8.0 and 8.5 per cent for garlic and ginger extract, respectively. The MIC of garlic observed in the present study was much less than that

of 8.8 per cent reported [13] which may be attributed to the change in the bacterial load and the strain variation. A study [23] revealed that the highest log CFU/g reduction values for $MIC_{90\%}$ assessed on minced meat inoculated experimentally with foodborne pathogen strains and against natural microbiota of meat were 1.3 and 1.0, respectively. The results was almost in accordance with that observed in a study [16], where it was reported that there was no antibacterial action for either garlic or ginger against *L. monocytogenes*.

The Gram positive bacteria were found to be more sensitive than gram negative bacteria for both spices, which is in agreement with the findings of other workers [24]. It may be related to the outer membrane of gram negative bacteria, which endows the bacterial surface with strong hydrophobicity and acts as a strong permeability barrier. This was in accordance with the invitro study [25], where the antibacterial activity of essential oils against *L. monocytogenes*, *Salmonella* Typhimurium, *E. coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus*, and *S. aureus* was evaluated and concluded that Gram-negative bacteria were less susceptible than Gram-positive bacteria.

In another study [26], it was observed that the cold aqueous extracts of garlic had inhibitory effects on *S. aureus* and *L. monocytogenes* bacterial isolates but selectively allowed the growth of *E. coli* when compared to the hot aqueous and ethanolic extracts while the hot aqueous extract of ginger had no effect on both organisms.

IV. CONCLUSION

Garlic and ginger extracts produced bacteriostatic effect against *Escherichia coli* and *Listeria monocytogenes* invitro. Both the spices exhibited more inhibitory effect against Gram positive organism as compared to Gram negative. Hence, both the spices provide a potential for their use as natural preservatives in food.

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Tables and Figures

Tables

Table 1. Antimicrobial effect of Garlic and Ginger extracts on *Escherichia coli*

Concentration of spices (%)	Mean OD value	
	Garlic	Ginger
0.5	0.574^{a1} ±0.023	0.457^{a2} ±0.023
1.0	0.558^{ab1}±0.023	0.296^{b2} ±0.034
2.0	0.440^{b1} ±0.051	0.267^{b2} ±0.028
4.0	0.213^{c1} ±0.040	0.185^{c1} ±0.017
7.0	0.168^{cd1}±0.051	0.106^{cd1}±0.017
10.0	0.078^{d1} ±0.032	0.063^{d1} ±0.017
Control 0.599±0.222		

P≤0.05

Figures bearing the same superscript in the column do not differ significantly. Figures bearing the same numerals in the row do not differ significantly

Table 2. Antimicrobial effect of Garlic and Ginger extracts on *Listeria monocytogenes*

Concentration of spices (%)	Mean OD value	
	Garlic	Ginger
0.5	0.384^{a1} ±0.023	0.459^{a2} ±0.023
1.0	0.330^{b1}±0.034	0.355^{b1} ±0.040
2.0	0.245^{c1} ±0.023	0.258^{c1} ±0.034
4.0	0.202^{cd1} ±0.011	0.195^{cd1} ±0.005
7.0	0.132^{d1}±0.040	0.154^{d1}±0.017
10.0	0.056^{e1} ±0.023	0.062^{e1} ±0.017
Control 0.599±0.222		

P≤0.05

Figures bearing the same superscript in the column do not differ significantly. Figures bearing the same numerals in the row do not differ significantly

Figures

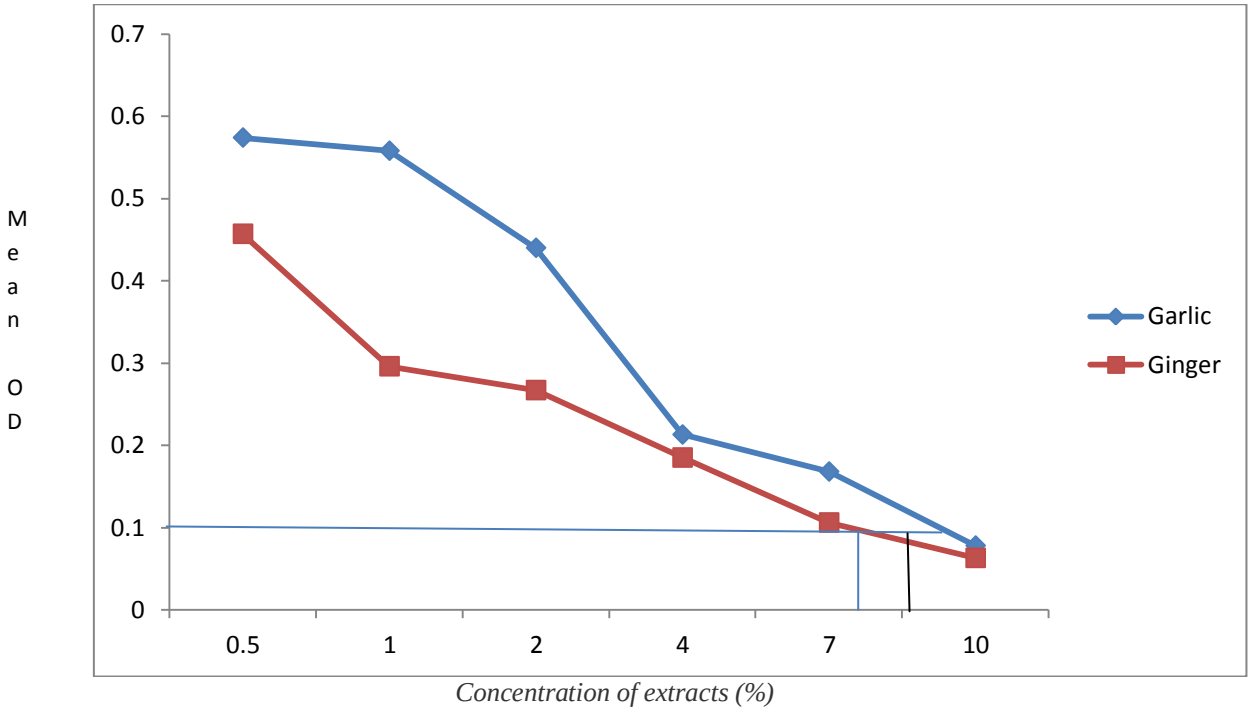

Fig. 1. Antimicrobial effect of Garlic and Ginger extracts on *Escherichia coli*

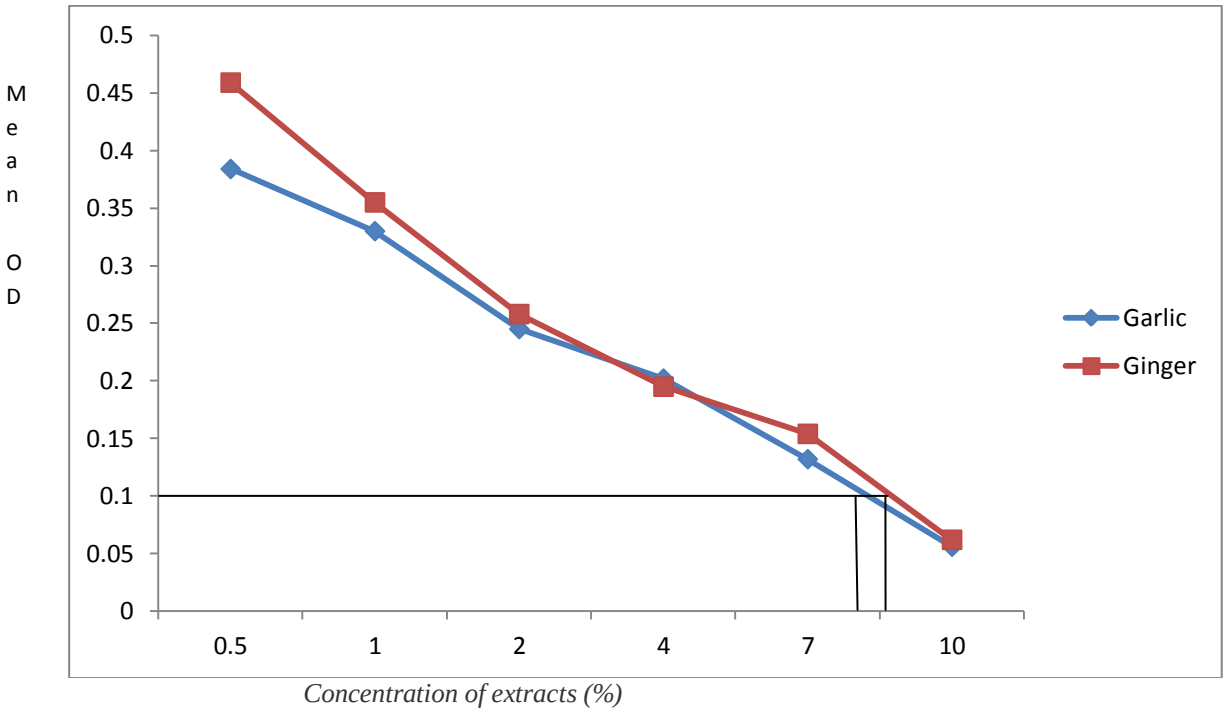

Fig. 2. Anti microbial effect of Garlic and Ginger extracts on *Listeria monocytogenes*

