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Comparison of antimicrobial activity of *Cinnamomum zeylanicum* and *Cinnamomum cassia* on food spoilage bacteria and water borne bacteria

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ABSTRACT

The objective of this study is to compare the antibacterial activity of the essential oil from bark of two cinnamon species, Cinnamomum zeylanicum and Cinnamomum cassia and their chemical constituents in order to develop an effective and economic food spoilage resistant spray. Their antibacterial activity is tested against food spoilage bacteria Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus. Escherichia coli is a food spoilage and water borne pathogen. Efficacy of the essential oil of Cinnamomum species was compared using Disc Diffusion method and MIC [1, 2] was calculated. Gentamycin, an anitibiotic, is used as a positive control. All three spoilage bacteria were found to be sensitive towards the essential oil of Cinnamomum species. However, the essential oil of Cinnamomum cassia was found to have more effective antimicrobial activity showing its maximum efficacy for E. coli.

Keywords: *Cinnamomum zeylanicum*, *Cinnamomum cassia*, Disc Diffusion Method, MIC (Minimum Inhibitory Concentration).

INTRODUCTION

In the recent years due to the increased susceptibility of fresh food towards the microbial stresses, it has become more important to develop certain techniques that can be effectively used against the fresh food spoiling microorganisms. Traditionally, the natural agents such as turmeric, garlic etc., have proved to be potential antimicrobial and antifungal agents. Thus we tried to evaluate the essential oil of different natural agents for its antimicrobial activity. The essential oil of aromatic plants and their components have a wide range of applications in ethno medicine, preservation, food flavouring, fragrances and perfume industries. Therefore, considerable attention has been focused on the various biological effects of these naturally occurring agents [3, 4, 5]. Among them is the cinnamon essential oil and its constituents which are known to possess various antifungal and antimicrobial activities [6, 7, 8]. Cinnamon, a spice, is commonly used in sweet and savoury food industry. Its oil is commonly used in the food industry because of its aroma [9]. Of all the available species of cinnamon we have focussed our studies on *Cinnamomum cassia* and *Cinnamomum zeylanicum.* Since these are widely grown in the regions of India, Sri Lanka and other south east countries which make them easily available to the local people. Also the comparison of these two species of Cinnamon i.e. *Cinnamomum zeylanicum* and *Cinnamomum cassia* to check their antimicrobial activity on *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as a food spoilage micro-organisms has not been explored yet.

The major component in *C. cassia* bark oil was found to be trans-cinnamaldehyde while in *C. zeylanicum* it was eugenol [10, 11, 12]. Its antimicrobial property is also attributed to the presence of cinnamyl acetate, methoxy cinnamaldehyde (MOCA) and other volatile compounds [13, 14].

The aim of this study is to assess the efficacy of the antimicrobial activity of essential oil from the bark of two common species of cinnamon, *Cinnamomum cassia* and *Cinnamomum zeylanicum*, against the food spoilage microorganism, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.* And finally to identify that among *Cinnamomum cassia* and *Cinnamomum zeylanicum*, the essential oil of which *Cinnamomum* species has the maximum efficacy so that the results can be further used to make an effective food spoilage resistant spray.

MATERIALS AND METHODS

Chemicals: All chemicals and reagents used were from Hi Media chemicals. Following chemicals were used - Mueller Hinton broth, Luria Bertani Broth, Dextrose, Methylene Blue, DMSO (Dimethyl sulfoxide), Gentamycin.

Sample preparation

Saline solution of concentration 0.9% w/v was prepared using sodium chloride (NaCl) and distilled water. Pure cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were taken and were serially diluted in 0.9% saline solution up to 10^{-5} dilution. Cinnamon bark oil of species *Cinnamomum cassia* and *Cinnamomum zeylanicum* were purchased from Kanpur, Uttar Pradesh, India. Different concentrations of oil of both the species of cinnamon were prepared in DMSO (Table 1). MHA-GMB (Mueller Hinton Agar - Glucose Methylene Blue) media was used for the inoculation of each type of microbe. 250 mL of MHA-GMB media was prepared using 5.5g of Mueller Hinton Broth, 2% dextrose, 2-3drops of methylene blue and 1% of agar. The antimicrobial disc of 6 mm diameter was prepared using Whatman filter paper with different concentrations of cinnamon oil as mentioned in table 1.

6 plates of MHA media were prepared which were then inoculated with 2 loops of inoculum of 10^{-5} dilution. Discs were prepared with 5 different concentrations of Cinnamon oil. These were then placed in the middle of plates already spread with inoculum.

Table 1: Preparation of Different Concentrations of Oil

S. No.	Concentration of oil (%)	Volume of DMSO (mL)	Volume of oil (mL)
1.	0.625	9.9375	0.0625
2.	1.25	9.875	0.125
3.	2.5	9.75	0.25
4.	5.0	9.5	0.5
5.	10.0	9.0	1.0

Measurement of Activity Index

Formula used,

Activity Index = (Zone of inhibition of extract / Zone of inhibition of antibiotic).

Zone of inhibition of stocks at different concentration of oil was measured and similarly zone of inhibition of antibiotic (Gentamycin) was measured.

RESULTS AND DISCUSSION

The antimicrobial activity of the two cinnamon species, *Cinnamomum zeylanicum* and *Cinnamomum cassia*, were compared on three food spoilage and water borne bacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

Analysis of the effect of the two cinnamon oil species was done after an incubation period of 24 hour at 40^{o} C.

The zone of inhibition for Gentamycin was measured (Table 2) and Activity Index of both species of Cinnamon for the gram negative- *Pseudomonas aeruginosa*, *Escherichia coli* and gram positive *Staphylococcus aureus* bacteria was calculated at different concentrations (Table 2, 3, 4).

Table2: Zone of Inhibition of Gentamycin

Name of Organism	Zone of Inhibition of Gentamycin (mm)
Pseudomonas aeruginosa	12
Staphylococcus aureus	41
Escherichia coli	22

Table 3: Effect of the oil of *C. zeylanicum* and *C. cassia* on *Pseudomonas aeruginosa* growth

S. No	Concentration of oil (%) v/v	*Cinnamomum cassia*		*Cinnamomum zeylanicum*	
		Zone of Inhibition (mm)	Activity Index	Zone of Inhibition (mm)	Activity Index
1.	0.0	0.0	0.0	0.0	0.0
2.	0.625	5.0	0.416	5.0	0.416
3.	1.25	7.0	0.583	6.0	0.500
4.	2.5	8.0	0.667	7.0	0.667
5.	5.0	9.0	0.750	8.0	0.750
6.	10.0	10.0	0.830	9.0	0.830

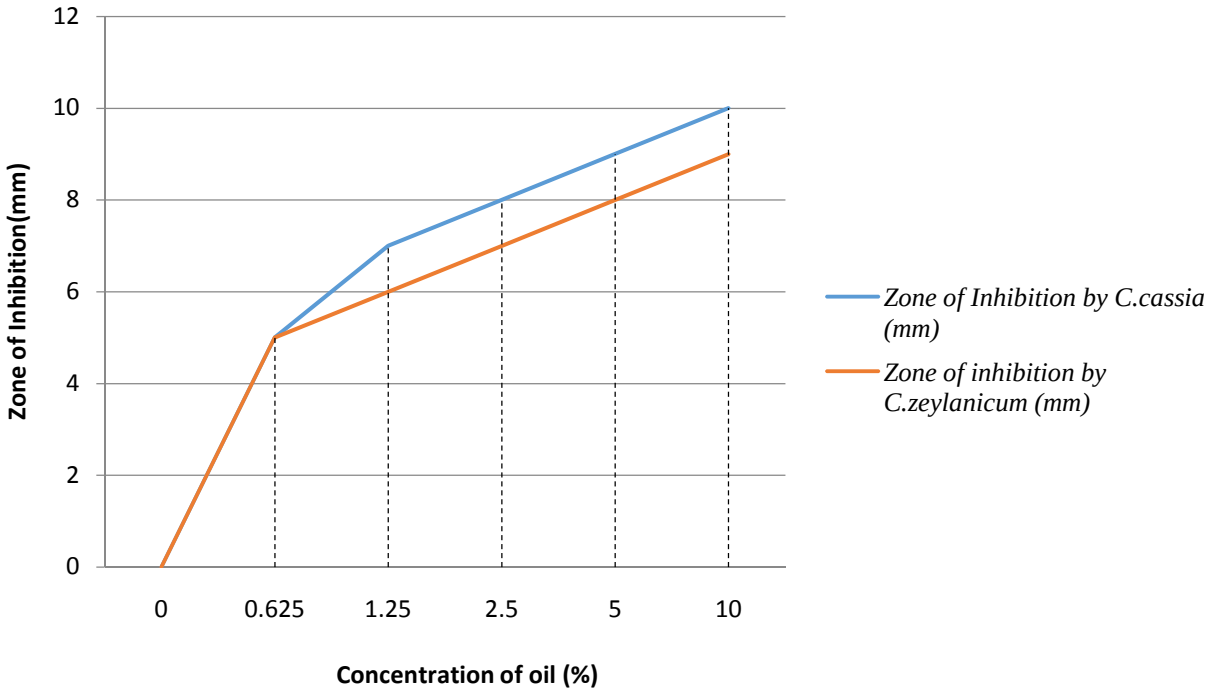

Figure 1: Zone of inhibition by *C. cassia* and *C. zeylanicum* against *P. aeruginosa* at different concentrations of cinnamon oil.

The result obtained for the effect of essential oils on *Pseudomonas aeruginosa* showed that at a concentration of 0.625% v/v, *Pseudomonas aeruginosa* have similar sensitivity for both essential oils. But with the increase in concentration of oils, the sensititvity increased for *C.cassia* showing maximum sensitivity at the 10% v/v.

Table 4: Effect of the oil of *C. zeylanicum* and *C. cassia* on *Staphylococcus aureus*

S.No	Concentration of oil (%) v/v	*Cinnamomum cassia*		*Cinnamomum zeylanicum*	
		Zone of Inhibition (mm)	Activity Index	Zone of Inhibition (mm)	Activity Index
1.	0.0	0.0	0.0	0.0	0.0
2.	0.625	8.0	0.190	7.0	0.170
3.	1.25	9.0	0.219	8.0	0.195
4.	2.5	10.0	0.243	9.0	0.219
5.	5.0	11.0	0.268	12.0	0.292
6.	10.0	21.5	0.524	15.5	0.378

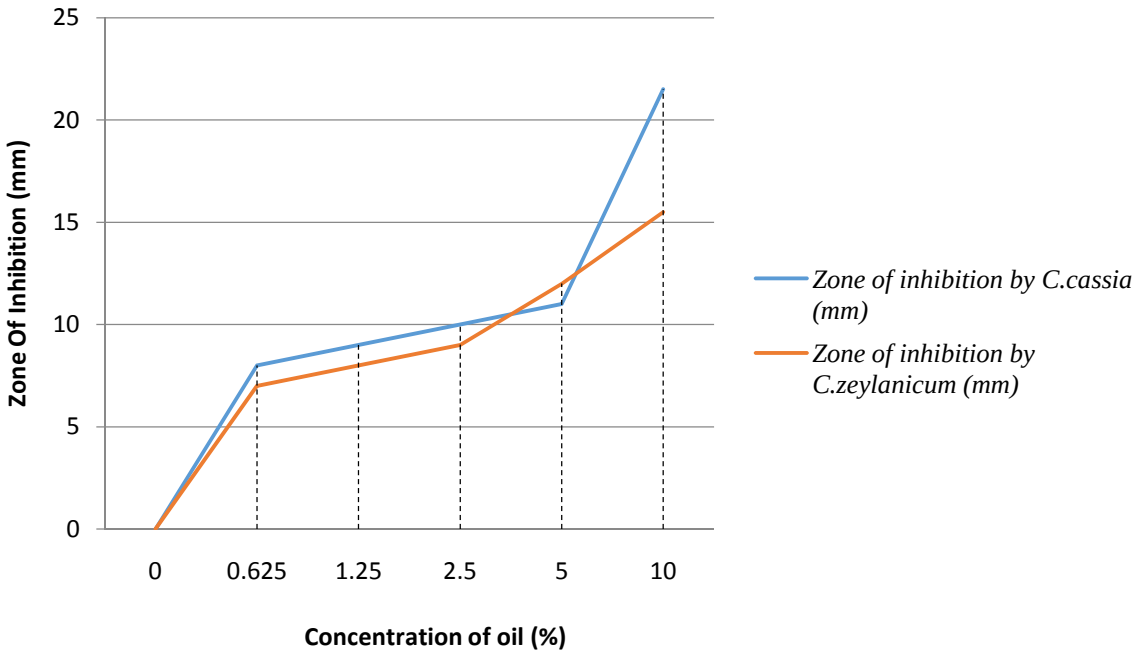

Figure 2: Zone of inhibition by *C. cassia* and *C. zeylanicum* against *S. aureus* at different concentrations of cinnamon oil.

For *S. aureus*, *C. cassia* showed higher activity both at lower (0.625%v/v) and higher (10% v/v) concentration of the essential oil. Although at 5%v/v concentration, the effect of *C. zeylanicum* was more but more importantly at the minimum effective concentration of 0.625%v/v, *Cinnamomum cassia* developed a larger zone of inhibition indicating its better efficacy at this concentration.

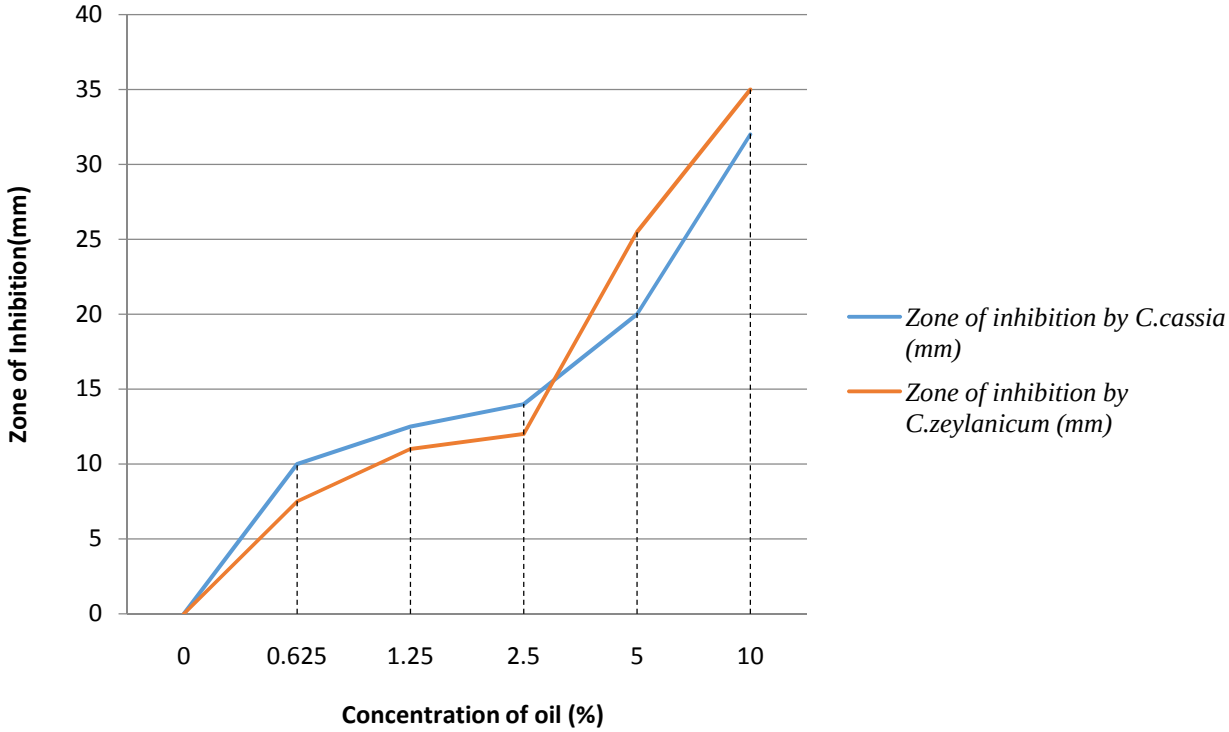

Figure 3: Zone of inhibition by *C. cassia* and *C. zeylanicum* against *E. coli* at different concentrations of cinnamon oil

Among the three bacteria tested, *E. coli* was found to be the most sensitive towards the antimicrobial activity of essential oils of C*innamomum* species. Unlike for *P. aeruginosa* and *S. aureus*, here *C. zeylanicum* developed bigger zone of inhibition at higher concentration. But at lower concentration of 0.625%v/v *C. cassia* still had more efficacy.

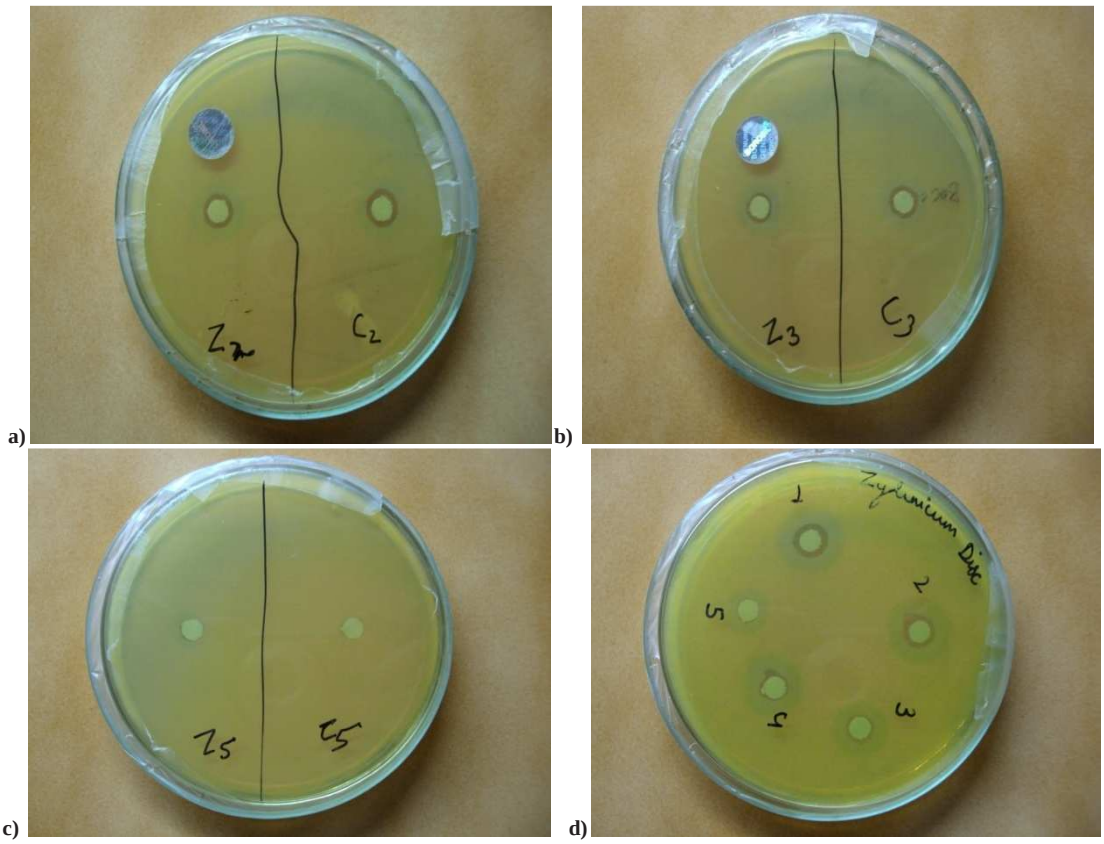

Figure 4. (a) Comparison of MIC of C. zeylanicum and C. cassia at 5% oil concentration (b) Comparison of MIC of C. zeylanicum and C. cassia at 2.5% oil concentration (c) Comparison of MIC of C. zeylanicum and C. cassia at 0.625% oil concentration (d) Comparison of MIC of C. zeylanicum for all oil concentrations

Table 5: Effect of the oil of *C. zeylanicum* and *C. cassia* on *Escherichia coli* growth

S.No	Concentration of oil (%) (v/v)	*Cinnamomum cassia*		*Cinnamomum zeylanicum*	
		Zone of Inhibition (mm)	Activity Index	Zone of Inhibition (mm)	Activity Index
1.	0.0	0.0	0.0	0.0	0.0
2.	0.625	10.0	0.454	7.5	0.340
3.	1.25	12.5	0.568	11.0	0.500
4.	2.5	14.0	0.636	12.0	0.545
5.	5.0	20.0	0.909	25.5	1.150
6.	10.0	32.0	1.450	35.0	1.590

Comparing all the three results, *C.cassia* was found to have a better antimicrobial activity at lower effective concentration of 0.625%v/v. Figure 6 and 7 clearly shows that among the three bacteria*E.coli* was found to be most susceptible against the action of cinnamon oil while *Pseudomonas aeruginosa* to be least.

The results of this work were found to be consistent with the work done by Hoque et al. (2007) who showed different effective concentration of essential oil of cinnamon against *Staphylococcus aureus* [15, 16], Friedman et al. (2002) who found that cinnamon oil was effective against *E. coli* [17] and Bowels et al. (1995) who showed that the essential oil of cinnamon inhibit the growth of *Staphylococcus aureus* [18]. These findings are also quite similar with the results of Chao et al. (2000) reporting that cinnamon bark oil fully inhibited the growth of some gram positive and gram negative bacteria, fungi and yeasts [19, 20]. As the main component, cinnamaldehyde, has proven to be particularly effective against some species of gram positive and gram negative bacteria including *Clostridium*, *Pseudomonas* and yeasts, *Candida* strains [21, 22, 23, 24]. It has been proposed that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of the bacteria [25, 26]. Therefore, the high antimicrobial activity of cinnamon oil is due to the presence of the high amount of cinnamaldehyde and due to high antibacterial activity of *C. cassia* ascertained by this study we can concur that cinnamaldehyde concentration is maximum in it, from the two species compared.

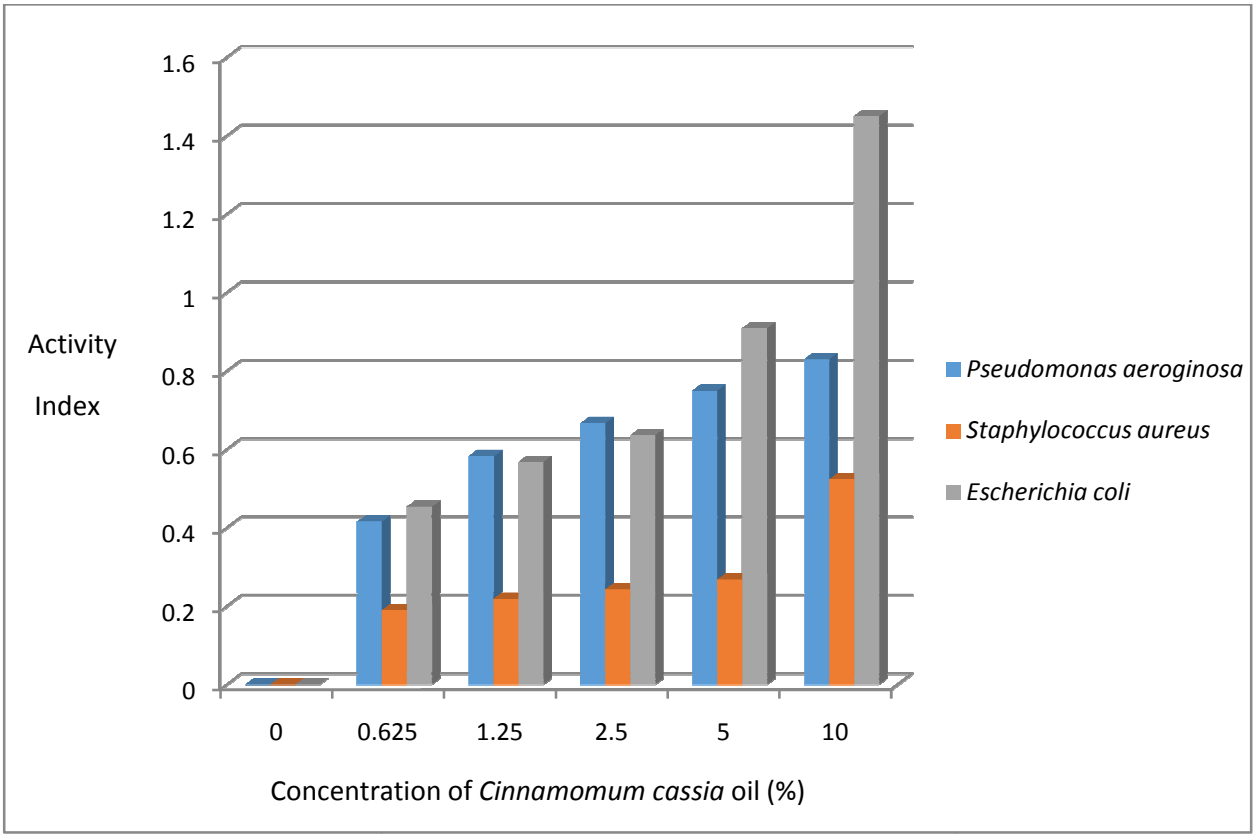

Figure 5: Susceptibility of micro organisms against *Cinnamomum cassia*

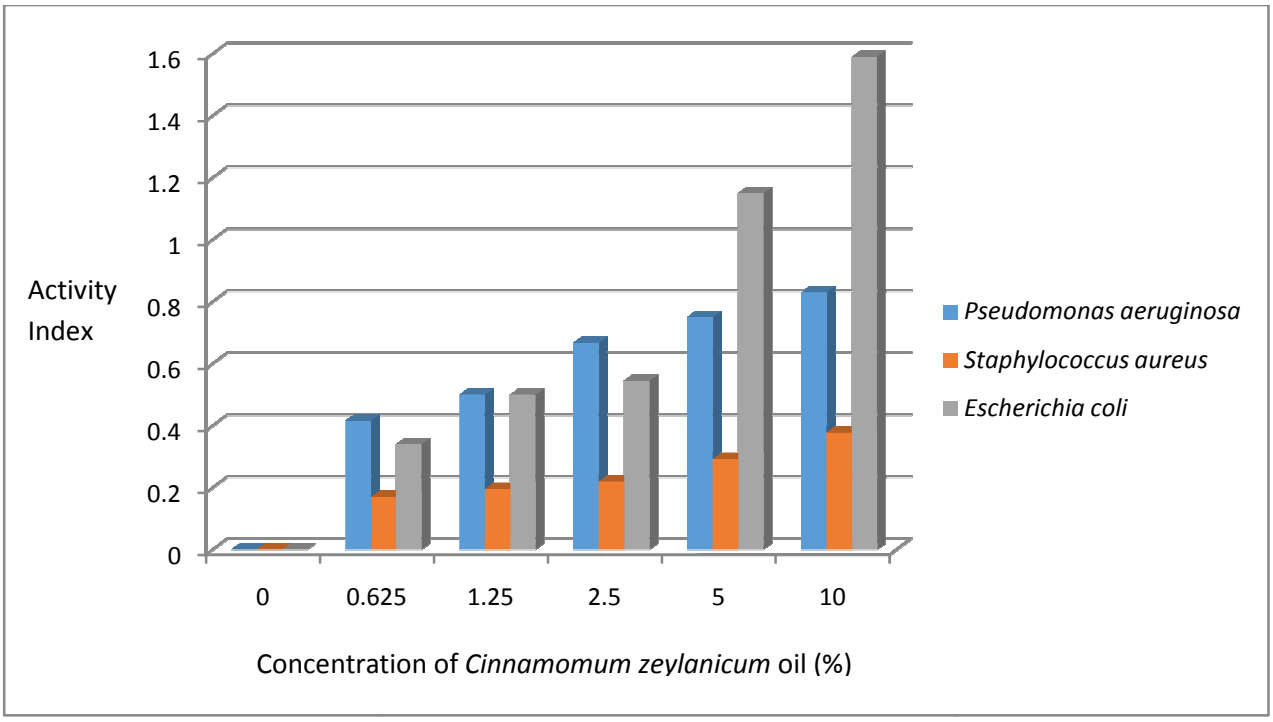

Figure 6: Susceptibility of microorganisms against *Cinnamomum zeylanicum*

CONCLUSION

By the present study it can thus be concluded that *Cinnamomum cassia* can be very successfully be used against the food spoiling bacteria *E. coli*. Coliform bacteria are easily encountered in water hence *C. cassia* can also be employed for limiting the spread of these bacteria through water or reducing their concentration minimal damaging limit. The minimum concentration required for *C. cassia* to act upon these spoilage bacteria was found to be 0.0625% v/v. Such a small concentration can be easily imparted in food products like apple juice (spoiled by *E. coli*), flavoured milk (spoiled by *Pseudomonas aeruginosa*) to inhibit the spoilage.

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