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*Full Length Research Paper*

# Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on somefood-borne microbes

**Charu Gupta[1*], Amar P. Garg[2], Ramesh C. Uniyal[1] and Archana Kumari[2]**

[1]Amity Institute for Herbal Research and Studies, Amity University Uttar Pradesh, Noida, U.P., India.
[2]Department of Microbiology, Chaudhary Charan Singh University, Meerut-250005, U.P., India.

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**The aim of the present study was to assess the antimicrobial activities of cinnamon (*Cinnamomum zeylanicum*) extract (50% ethanol) and its oil, and to compare their effectiveness against ten bacteria (seven Gram-positive and three Gram-negative) and seven fungi by agar well diffusion assays. Cinnamon oil exhibited a broad spectrum of antagonistic activity, as compared to its extract, by inhibiting both bacteria and fungi. The oil was found to be very effective with a lowest minimum inhibitory concentration (MIC) of 1.25% (v/v) against *Bacillus* sp., *Listeria monocytogenes, E. coli* and *Klebsiella* sp. Amongst the fungi, *Rhizomucor* sp. was found to be highly sensitive to the oil. Therefore, this study shows that cinnamon oil is a more potent antimicrobial agent than cinnamon extract and that it has the potential to be used as food biopreservative.**

**Key words:** Antimicrobial, cinnamon, biopreservative, food borne pathogens, agar well diffusion, essential oil.

## INTRODUCTION

There has been a constant increase in the search of alternative and efficient compounds for food preservation, aimed at partial or total replacement of antimicrobial chemical additives. Gould (1995) has emphasized the possible use of spices and their derivatives as alternatives for inclusion in a new perspective of food conservation called “natural antimicrobial system”, which relies on the synergistic effect of antimicrobial compounds from animal, plant and/or microbial origin in order to create an unfavourable environment for microbial survival in foods. The chief significance of the above study was therefore to test the antimicrobial activity of cinnamon oil and extract as an alternatives to chemical preservatives so as to minimize their side-effects and simultaneously improving the shelf-life of the food products. In the present study, we have compared the antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*) oil and its crude extract for the first time.

*Corresponding author. E-mail: cgupta@amity.edu.

## MATERIALS AND METHODS

### Materials

All chemicals used were of analytical reagent-grade and obtained from E. Merck (Mumbai, India). Cinnamon bark and ready-made cinnamon oil (*C. zeylanicum)* was collected from the local market in Meerut (Uttar Pradesh, India). Dr. C.M. Govil of the Botany Department, CCS University, Meerut, India, confirmed the plant species and the oil.

### Bacterial and fungal test isolates

Ten bacterial isolates (seven Gram-positive and three Gram-negative), mostly food-borne pathogens, were selected for this study. The Gram-positive bacteria comprised *Bacillus cereus, Bacillus subtilis, Bacillus* sp., *Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes* and *Micrococcus luteus*, while the Gram-negative bacteria comprised *Escherichia coli, Klebsiella* sp. and *Pseudomonas aeruginosa*. The fungal isolates used in this study were *Alternaria* sp., *Aspergillus fumigatus, Aspergillus niger, Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Rhizomucor* sp. The bacterial and fungal cultures were obtained from the Department of Microbiology, CCS University. The bacteria and fungi were cultured on nutrient agar medium and Sabouraud’s

dextrose agar (SDA) medium (Hi-Media, Mumbai, India), respectively. The agar plates were incubated at 37°C for 24 h (bacteria) and at 28°C for 3 days (fungi). The plates were stored at 4°C until required for sensitivity testing.

**Collection and processing of herbal sample**

The cinnamon bark was grounded in a milling machine (Inalsa Mixer Grinder) in order to obtain a fine dry powder. The powder was weighed, using a single pan electronic weighing balance (Ohaus), and the cinnamon extract was obtained by means of a maceration process. The cinnamon powder was soaked in 50% ethanol (1 g of powder per 5 ml of solvent) in a 250 ml Erlenmeyer flask for a period of 48 h at room temperature with frequent shaking. The flasks were closed with a cotton plug and aluminium foil. The mixture was then centrifuged at 3,500 × *g* for 20 min and finally filtered through Whatmann filter paper No.1 (Azoro, 2000). The filtrate was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Buchi) until a semi-solid substance was obtained, which was then dried in a crucible under a controlled temperature (45ºC) to obtain a solid powder (Jonathan and Fasidi, 2003). The process of extraction was repeated until 500 mg of the powder was obtained. The powder was weighed, reconstituted in dimethyl sulfoxide (DMSO) and stored at 4ºC. Once the extracts are dissolved in pure DMSO, these are also sterilized, and thus, a very costly and time-consuming step of membrane filtration sterilization was omitted (Zgoda and Porter, 2001).

**Determination of antimicrobial activity of cinnamon extract and oil**

The antimicrobial activities of cinnamon extract and oil were determined by the agar well diffusion method (Okeke et al., 2001). Briefly, each bacterium was first subcultured in nutrient broth at 37ºC for 24 h. One hundred microlitres (100 µl) of standardized inoculum ($10^6$ CFU/ml; 0.5 MacFarland) of each test bacterium was spread with the help of sterile spreader onto sterile Muller-Hinton Agar (MHA) (Hi-Media) so as to achieve confluent growth. The plates were allowed to dry and a sterile cork borer (6 mm diameter) was used to bore wells in the agar. Subsequently, a 50 µl volume of the extract and the oil was introduced in triplicate wells of the agar plates. Sterile DMSO served as negative control. A positive control in the form of sodium propionate (standard food preservative) was also included in the study. The plates were allowed to stand for at least 1 h for diffusion to take place and then incubated at 37°C for 24 h. The zone of inhibition was recorded to the nearest size in mm (Norrel and Messely, 1997).

**Determination of antifungal activity of cinnamon extract and oil**

For determining the antifungal activity of the cinnamon extract and oil, the fungal isolates were subcultured on SDA at 28ºC for 3 - 4 days. Sterilized Sabouraud's Dextrose Agar plates were taken and a sterile cork borer (6-mm diameter) was used to bore wells in the agar. A 50 µl volume of the extract and oil was introduced into each of the peripheral wells, while a fungal disc was inoculated into the central well. A negative control (sterilized DMSO) was also included in one of the peripheral wells to compare the activity. The plates were then incubated at 28ºC. The evaluations were carried out by means of daily measurement of the colony diameter, starting at 24 h after the experiment began and finishing when two-thirds of the plate surface of the control treatment was covered by the fungus (Fiori et al., 2000). The appearance of zones of inhibition was regarded as positive for the presence of antimicrobial action in the test substance.

The results were expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9 – 12 mm, partially active; 13 – 18 mm, active; >18 mm, very active (Junior and Zanil, 2000).

**Determination of minimum inhibitory concentration (MIC) of the extract and oil**

The MIC was defined as the lowest concentration that completely inhibited the growth for 24 h (Thongson et al., 2004). The MIC for the crude extract was determined by the agar well diffusion method. A two-fold serial dilution of the cinnamon extract and sodium propionate was prepared by first reconstituting it in DMSO. It was then diluted in sterile DMSO to achieve a decreasing concentration range of 500 to 62.5 mg/ml. For cinnamon oil, a decreasing concentration range of 10 to 0.625% (v/v) was prepared. A 50 µl volume of each dilution was added aseptically into the wells in Mueller Hinton agar plates that had been inoculated with standardized inoculums ($10^6$ CFU/ml) of the test bacteria. The agar plates were incubated at 37°C for 24 h. Sodium propionate only served as positive control. All experiments were performed in triplicate. The same procedure was used for fungi, except that SDA plates were used and the plates were incubated at 28°C. The lowest concentration of cinnamon extract and oil showing a clear zone of inhibition was considered as the MIC.

## RESULTS

### Inhibitory activity against bacteria

Cinnamon extract was found to be effective against almost all of the food-borne microbes. *B. cereus* was found to be the most sensitive to cinnamon extract with an inhibition zone diameter (IZD) of 17 mm, followed by *S. aureus* (16 mm). *Bacillus* sp., *B. subtilis* and *S. aureus* were found to be partially sensitive to the test extract with an IZD of 14, 14 and 13 mm, respectively. However, *P. aeruginosa* was found to be resistant, as shown in Table 1. While cinnamon oil inhibited the growth of all the test bacteria, it produced the widest IZD against *B. cereus* (29 mm), followed by *S. aureus* (20 mm). It also inhibited the growth of *P. aeruginosa* and produced an IZD of 16 mm (Table 1). The other test bacteria were also sensitive to the extract. Sodium propionate (standard food preservative) was found to be very effective against *B. cereus* with an IZD of 20 mm. The MIC values of cinnamon extract and sodium propionate (positive control) ranged between 62.5 - 1000 mg/ml, while that of cinnamon oil ranged between 1.25 - 5% (v/v). Cinnamon extract was found to be most effective with the lowest MIC of 62.5 mg/ml against *Bacillus* sp. and *S. aureus* (Table 2). While cinnamon oil inhibited the growth of both groups of bacteria (Gram-positive and Gram-negative), *Bacillus* sp., *L. monocytogenes*, *E. coli* and *Klebsiella* sp. had the lowest MIC of 1.25% (v/v) in each case (Table 2). *Bacillus* sp. was found to be very sensitive to sodium propionate and had the lowest MIC (62.5 mg/ml).

### Inhibitory activity against fungi

With regard to antifungal activity, the cinnamon extract

**Table 1.** Zone of inhibition (mm) of cinnamon extract and cinnamon oil against test bacteria on Mueller-Hinton agar medium

| Test bacterial isolates | Cinnamon extract | Cinnamon oil | Positive control | Negative control |
|---|---|---|---|---|
| *Bacillus cereus* | 17.0 | 29.0 | 20.0 | 0.0 |
| *Bacillus subtilis* | 14.0 | 16.0 | 14.0 | 0.0 |
| *Bacillius* sp. | 14.0 | 15.0 | 14.0 | 0.0 |
| *Staphylococcus aureus* | 16.0 | 20.0 | 17.0 | 0.0 |
| *Staphylococcus epidermidis* | 13.0 | 15.0 | 14.0 | 0.0 |
| *Listeria monocytogenes* | 10.0 | 18.0 | 12.0 | 0.0 |
| *Micrococcus luteus* | 15.0 | 18.0 | 14.0 | 0.0 |
| *Escherichia coli* | 11.0 | 16.0 | 12.0 | 0.0 |
| *Klebsiella* sp. | 11.0 | 14.0 | 12.0 | 0.0 |
| *Pseudomonas aeruginosa* | 0.0 | 16.0 | 11.0 | 0.0 |

Negative control- Dimethyl sulfoxide; Positive control- Sodium propionate. Each value is the average of three independent replicates.

**Table 2.** The MIC values of cinnamon extract (mg/ml) and cinnamon oil (% [v/v]) on Mueller-Hinton agar medium

| Test bacterial species | Cinnamon extract (mg/ml) | Cinnamon oil(% [v/v]) | Sodium propionate (mg/ml) |
|---|---|---|---|
| *Bacillus cereus* | 250 | 2.5% | 125 |
| *Bacillus subtilis* | 500 | 2.5% | 250 |
| *Bacillius* sp. | 62.5 | 1.25% | 62.5 |
| *Staphylococcus aureus* | 62.5 | 2.5% | 125 |
| *Staphylococcus epidermidis* | 500 | 2.5% | 500 |
| *Listeria monocytogenes* | 500 | 1.25% | 500 |
| *Micrococcus luteus* | 500 | 5% | 500 |
| *Escherichia coli* | 1000 | 1.25% | 500 |
| *Klebsiella* sp. | 1000 | 1.25% | 500 |
| *Pseudomonas aeruginosa* | - | 5% | 1000 |

Each value is the average of three independent replicates.

**Table 3.** Zone of inhibition (mm) of cinnamon extract and cinnamon oil against common food spoilage fungi on SDA medium

| Test fungal species | Cinnamon extract | Cinnamon oil | Positive control | Negative control |
|---|---|---|---|---|
| *Alternaria* sp. | 25.0 | 38.0 | 26.0 | 0.0 |
| *Aspergillus fumigatus* | 15.0 | 38.0 | 18.0 | 0.0 |
| *Aspergillus niger* | 0.0 | 29.0 | 15.0 | 0.0 |
| *Aspergillus* sp. | 10.0 | 24.0 | 16.0 | 0.0 |
| *Penicillium* sp. | 35.0 | 35.0 | 32.0 | 0.0 |
| *Rhizopus* sp. | 0.0 | 26.0 | 18.0 | 0.0 |
| *Rhizomucor* sp. | 10.0 | 40.0 | 20.0 | 0.0 |

Negative control- Dimethyl sulfoxide; Positive control- Sodium propionate. Each value is the average of three independent replicates.

resulted in the widest IZD against *Penicillium* sp. (35 mm), but was inactive against *A. niger* and *Rhizopus* sp. (Table 3). Similarly, cinnamon oil inhibited the growth of all test fungi. The widest IZD was produced against *Rhizomucor* sp. (40 mm), followed by *Alternaria* sp. and *A. fumigatus* with an IZD of 38 mm each (Table 3). Sodium propionate demonstrated a moderate spectrum of activity against all test fungi. *Penicillium* sp. was found to be highly sensitive to sodium propionate with an IZD of 32 mm (Table 3).

For the fungi, the MIC values of cinnamon extract, oil and sodium propionate exhibited a broad range. *Penicil-*

**Table 4.** The MIC values of cinnamon extract (mg/ml) and cinnamon oil in (% [v/v]) against test fungi on SDA medium

| Test fungal species | Cinnamon extract (mg/ml) | Cinnamon oil (% [v/v]) | Sodium propionate (mg/ml) |
|---|---|---|---|
| *Alternaria sp.* | 4000 | 5 | 1000 |
| *Aspergillus fumigatus* | - | 5 | 4000 |
| *Aspergillus niger* | - | 10 | 4000 |
| *Aspergillus sp.* | 8000 | 10 | 2000 |
| *Penicillium sp.* | 2000 | 5 | 500 |
| *Rhizopus sp.* | - | 10 | 1000 |
| *Rhizomucor sp.* | 8000 | 2.5 | 1000 |

Each value is the average of three independent replicates.

*lium* sp. was found to be highly sensitive to the cinnamon extract by showing the lowest MIC of 2000 mg/ml, while the extract was ineffective against *A. fumigatus, A. niger* and *Rhizopus sp.* as shown Table 4. For sodium propionate the MIC values ranged between 500 - 4000 mg/ml. Thus, sodium propionate was found to be more effective as an antifungal agent compared to the cinnamon extract. In contrast, cinnamon oil was active against all the test fungi and the MIC values ranged between 2.5 to 10% (v/v), with the lowest MIC of 2.5% (v/v) against *Rhizomucor sp.* as shown Table 4.

## DISCUSSION

The activity of cinnamon is due to the presence of cinnamaldehyde, an aromatic aldehyde that inhibits amino acid decarboxylase activity (Wendakoon and Sakaguchi, 1995), and has been proven to be active against many pathogenic bacteria (Suresh et al., 1992). Cinnamon bark is rich in cinnamaldehyde (50.5%), which is highly electro-negative. Such electro-negative compounds interfere in biological processes involving electron transfer and react with nitrogen-containing components, e.g. proteins and nucleic acids, and therefore inhibit the growth of the microorganisms.

Cinnamon oil contains benzoic acid, benzaldehyde and cinnamic acid, of which the lipophylic moiety of these compounds has been recognized as being responsible for its antimicrobial property (Ramos-Nino et al., 1996). Also, cinnamon oil from bark contains 4.7% eugenol (Ranasinghe et al., 2002). Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used (Pelczar et al., 1988). These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data (Charai et al., 1996; Sivropoulou et al., 1996; Hili et al., 1997; Lis-Balchin and Deans, 1997). Essential oil from cinnamon bark also contains cinnamyl acetate (8.7%), which increases the activity of the parent compound.

The fungistatic or fungicidal effect of cinnamon spice and its oil is due to the inhibitory action of natural products. The mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly, culminating with mycelium germination inhibition (Cowan, 1999). Also, it has been reported that plant lytic enzymes act on the fungal cell wall, causing breakage of β-1,3 glycan, β-1,6 glycan and chitin polymers (Brull and Coote, 1999).

## CONCLUSION

In conclusion, cinnamon oil was found to be a much better antagonistic agent, exhibiting broad range of antimicrobial activity against common bacteria and fungi than its extract and sodium propionate. Hence, it represents an alternative source of natural antimicrobial substances for use in food systems to prevent the growth of food-borne bacteria and extend the shelf-life of the processed food. The study also shows that further research on the effects of spices and essential oils on microorganisms can be rewarding to pursue in the search for new broad-spectrum antimicrobial agents.

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