NOTE

Antibacterial activity of *Cinnamomum zeylanicum* fruit extracts

P. S. Negi, G. K. Jayaprakasha* and L. Jaganmohan Rao

SUMMARY

*Cinnamomum zeylanicum* Blume, the cinnamon of commerce, provides various types of oils depending on the part of the plant distilled. At present, cinnamon fruits are not being used for the production of volatile oils, also no report is available on its biological activities. Cinnamon fruits were powdered and extracted with benzene, ethyl acetate (EtOAc), methanol and water for 8 h each. The extracts were filtered, concentrated and dried under vacuum. Antibacterial activity of the above extracts were evaluated using pour plate method at 250, 500 and 1000 ppm concentrations against *Bacillus cereus*, *B. coagulans*, *B. subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. All the crude extracts showed a broad spectrum of antibacterial activity. EtOAc and benzene extract showed higher antibacterial activity than methanol and water extracts.

Keywords

*Cinnamomum zeylanicum*, antibacterial activity, *Bacillus cereus*, *B. coagulans*, *B. subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

1 – INTRODUCTION

The spoilage and poisoning of foods by microorganisms are the problems that have not yet been brought under adequate control despite the range of robust preservation techniques available. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin. Therefore, natural alternatives are needed to achieve sufficient long shelf life and high degree of safety with respect to food borne pathogenic microorganisms. In nature, there are a large number of antimicrobial compounds, which can have a potential application in the control of microbial spoilage of foods (HSIEH et al., 2001).

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Volatile oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal and antioxidant properties (Deans et al., 1993). With the growing interest in the use of volatile oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for these properties has become increasingly important. The use of natural antimicrobial compounds is important not only in the preservation of food, but also in the control of human and plant diseases of microbial origin. Bacterial and fungal infections pose a greater threat to health, most notably in immunocompromised subjects; hence the need of hour is to find a cheap and effective antimicrobial agents (Baratta et al., 1998).

Cinnamomum zeylanicum Blume, the cinnamon is native to Sri Lanka and tropical Asia. The tree occurs in South India up to altitudes of 500 meters, but mostly grown below 200 meters. The tree flowers in January and flowers ripen during May-August (The Wealth of India, 1992). Cinnamon is a rich source for essential oils and tannins, which are the major constituents inhibiting microbial growth (Bullerman et al., 1977; Chang, 1995). Thirty-four compounds have been previously identified in cinnamon fruit oil with (E)-cinnamyl acetate (42-54%) and (E)-caryophyllene (9-14%) as the major components (Jayaprakash et al., 1997). Literature survey revealed that no reports exist on antibacterial activity of cinnamon fruits. Hence, we report its antibacterial activity against a few Gram-positive and Gram-negative bacteria in order to establish the biological activity of this unconventional part of cinnamon for the use in food preservation and pharmaceutical applications.

2 – MATERIALS AND METHODS

2.1 Materials

Cinnamomum zeylanicum Blume (synonym C. verum J. S. Presl) fruits were collected from Karkala (Karnataka, India). The species was identified, and a voucher specimen was deposited at Manasagangotri herbarium (MGH No. 2/96/01), Department of Botany, University of Mysore, Mysore, India. All the solvents and chemicals used were of AR grade.

2.2 Extraction

Air-dried cinnamon fruits (100 g) were powdered to 40-60 mesh and extracted in a Soxhlet extractor with benzene, ethyl acetate (EtOAc), methanol and water for 8 h each separately. The extracts filtered and concentrated under vacuum to get the yield of 0.78, 1.87, 4.99 and 3.87 g (w/w), respectively.

2.3 Samples preparation

Benzene, ethyl acetate, methanol and water extracts (250 mg each) were dissolved in propylene glycol and transferred to 10 ml volumetric flasks separately and made up to the mark.
2.4 Preparation of agar medium

Nutrient agar (13 g) (HiMedia, Mumbai, India) was dissolved in water 1000 ml distilled water and 20 ml aliquots of agar medium were transferred into 100 ml conical flasks and sterilized at 121°C for 20 min in an autoclave.

2.5 Microorganisms and culture media

Strains of *Bacillus cereus* (F4810), *B. coagulans* (CFR1681), *B. subtilis* (CFR1604), *Staphylococcus aureus* (FRI722), *Escherichia coli* (D21) and *Pseudomonas aeruginosa* (CFR1704) were obtained from the stock culture collection of Food Microbiology Department, CFTRI, Mysore. The bacterial cultures were maintained at 4°C on nutrient agar slants and sub-cultured at 15 days intervals. Prior to use, the cultures were grown in nutrient broth (HiMedia, Mumbai, India) at 37°C for 24 h. A pre-culture was prepared by transferring 1 ml of this culture to 9 ml nutrient broth and cultivated for 48 h at 37°C. The cells were harvested by centrifugation at 1200 x g for 5 min, washed and suspended in saline.

2.6 Antibacterial activity

The cinnamon extracts were tested against different micro-organisms as per the method of NEGJ et al. (1999). To flasks containing 20 ml of melted nutrient agar, different concentrations (250, 500 and 1000 ppm) of test material were added. In case of control, equivalent amount of propylene glycol was added. One hundred μl (about 10^3 cfu/ml) of each bacterium to be tested was inoculated into the flasks under aseptic conditions. The contents were mixed thoroughly and media was then poured into sterilized petri plates in quadruplet and incubated at 37°C for 20-24 h. The colonies developed after incubation were counted and expressed as colony forming units per ml of culture (cfu/ml). The inhibitory effect was calculated using the following formula: % Inhibition = (1 - T/C) x 100, where T is cfu/ml of test sample and C is cfu/ml of control.

For minimum inhibitory concentration (MIC) determination different concentration of extracts (250-1500 ppm) were tested against each bacterium, and MIC was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested.

2.7 Statistical analysis

The MIC values were analysed by two way ANOVA without replication to compare the effect of different extracts and bacteria. The Duncun’s Multiple Range Test (DMRT) was used for making comparisons among different values (GOMEZ and GOMEZ 1984).
3 – RESULTS AND DISCUSSIONS

The benzene, ethyl acetate, methanol and water extracts of cinnamon fruits showed variable antibacterial activity (table 1). EtOAc extract was most effective against all the bacteria tested. *B. cereus* and *P. aeruginosa* were inhibited completely by this extract at 250 ppm, and 1400 ppm were required for complete inhibition of *E. coli* showing their least and highest resistance towards the EtOAc extract. Methanol and water extracts were least effective against all the bacteria tested. Growth of none of the organisms was inhibited completely by these extracts even at 500 ppm except for *B. coagulans* by methanol extract. Benzene extract showed statistically similar activity to EtOAc extract, and inhibited complete growth of *B. cereus, B. subtilis, B. coagulans* and *P. aeruginosa* at or below 500 ppm.

Table 1
Minimum Inhibitory Concentration of *Cinnamomum zeylanicum* fruit extracts (ppm)*.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Benzene extract</th>
<th>EtOAc extract</th>
<th>MeOH extract</th>
<th>water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>300&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>700&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>750&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>500&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>400&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>800&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>B. coagulans</em></td>
<td>400&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>300&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>500&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>600&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>500&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>750&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>800&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>400&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>700&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>750&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1500&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1400&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1500&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1500&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* result of 4 replications of same concentration where no growth was observed; values followed by same letter are not significantly different (p < 0.05).

The above observations were confirmed when the growth inhibition of bacteria were compared at fixed concentration of various extracts. The statistical analysis showed that MIC was significantly (p < 0.05) affected by both extracts and bacteria. The MIC values of EtOAc extract were minimum showing its highest antibacterial activity, which was statistically similar to benzene extract. The variable activity observed among different extracts may be due to the differences in active compounds extracted by different solvents. Although various compounds present in cinnamon leaf and bark essential oil are reported to be biologically active (Saksema and Saksema, 1984; Tiwari et al., 1994; Nakamura et al., 1990; Mallavarapu et al., 1995), the active components present in cinnamon fruit are yet to be identified. Earlier, Negi and Jayaprakash (2001, 2003) reported variable activity of compounds extracted by different solvents from grapefruit and pomegranate peels.

In general, Gram-negative bacteria are reported to be more resistance to external agents as their outer membrane acts as permeability barrier due to the presence of lipopolysaccharides which makes them inherently resistant to antibiotics, detergent and hydrophilic dyes (NiKaido and Vaara 1985). Gram-positive bacteria contain an outer peptidoglycan layer, an ineffective permeability barrier
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in their cell wall, which makes them sensitive to external environment (SCHERRER et GERHARDT 1971). In this study, all the Gram positive bacteria and E. coli, a Gram negative bacterium showed similar observation, but the other Gram negative bacterium P. aeruginosa behaved differently and showed similar activity as Gram positive bacteria.

4 – CONCLUSIONS

The results shown above indicated that the EtOAc and Benzene extracts has high antibacterial activities. The antibacterial properties of cinnamon fruits can add the value to this wonder plant, as at present except fruits all parts of this plant are being utilized. This is the preliminary report on the isolation of antibacterial fractions from cinnamon fruits, further studies are required to isolate and identify the bioactive compounds. The relationship between antibacterial activity and chemical composition of the extract is a very interesting area for future research.

5 – ACKNOWLEDGEMENTS

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REFERENCES


