Effects of Aqueous Extract of *Cinnamomum zeylanicum* on Pathogenic Gastrointestinal Bacteria and Probiotic Bacteria

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Abstract

**Background:** Bacterial resistance to antibiotics and the progress made in medicinal plants have caused researchers to pay attention to antibacterial compounds in plants.

**Objectives:** In this study, we assessed the prebiotic and antipathogenic effects of the aqueous extract of *Cinnamomum zeylanicum*.

**Materials and Methods:** Antibacterial activity of the aqueous extract was performed on various target cultures. Determining the activity of the extract in inhibiting the growth of bacteria was done using broth dilution technique. We evaluated the prebiotic activity of extract on *Lactobacillus reuteri* and *Lactobacillus acidophilus* probiotic bacteria.

**Results:** *C. zeylanicum* extract was able to inhibit the growth of all six pathogens. Furthermore, *C. zeylanicum* extract had prebiotic properties for *L. reuteri* and *L. acidophilus* bacteria.

**Conclusion:** There are antibacterial substances in the aqueous extract of *C. zeylanicum*, and it has prebiotic activity. Therefore, this makes *C. zeylanicum* considered a good candidate for food supplementation to prevent gastroenteritis.

**Keywords:** *Cinnamomum zeylanicum*, Antibacterial activity, Prebiotic, Probiotic

**Background**

One of the important diseases that humans have always been dealing with is bacterial disease. Resistance to antibiotics and changes in the normal gut flora are important issues in the treatment of infectious diseases. Furthermore, other side effects of antibiotics such as skin rashes, hives, joint pain, weakness, lethargy, severe anaphylactic shock, and hemolytic anemia have drawn the attention of researchers to find new effective antimicrobial compounds against pathogens.

Plants can synthesize various secondary metabolites that are produced in response to pathogens or stress. The use of plants with antimicrobial effect is considered in medicine. In addition to the antimicrobial effects of some plants, they may play a prebiotic role in improving the number of probiotic bacteria. Prebiotics are indigestible fermented substances that selectively stimulate the growth, composition, and activity of the gut microflora in the digestive system, and therefore, increase and improve the health of the host.

Lactic acid bacteria are known as a large group of probiotic bacteria. They can tolerate bile salts and stomach acidity. These microorganisms can be a part of the gut microflora with the ability to bind to intestinal cells and compete with adherence to pathogens. They can also produce antimicrobial compounds such as lactic acid and bacteriocin.

Plants with anti-pathogenic properties and prebiotic activity have drawn the attention of researchers. *Cinnamomum zeylanicum* belongs to *Lactobacillus auraceae* family. *C. zeylanicum* bark is used in the treatment of various diseases such as diarrhea, indigestion, flatulence, flu, and bronchitis, controlling infections and reducing blood sugar levels in diabetic patients. In addition, the anti-inflammatory, anti-mitotic, and anti-cancer properties of *C. zeylanicum* are important. There is evidence indicating that *C. zeylanicum* has no side effects and can play a role in improving the health of patients as an adjunctive treatment.

This study was designed to investigate the antibacterial activity of *C. zeylanicum* bark extract against *Escherichia coli*, *Salmonella Typhi*, *Staphylococcus aureus*, *Clostridium difficile*, and *Pseudomonas aeruginosa* pathogens and the effect of this extract on the growth of *Lactobacillus acidophilus* and *Lactobacillus reuteri* bacteria.

**Materials and Methods**

**Bacterial Strains and Culture Conditions**

Two probiotic bacteria (*L. acidophilus* and *L. reuteri* protectis) and five pathogenic bacteria (*enterohemorrhagic*...
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E. coli PTCC 1399, S. Typhi PTCC 1609, S. aureus PTCC 1431, C. difficile, PTCC 1765, and P. aeruginosa PTCC 1707) were purchased from the Persian Type Culture Collection center. The appropriate media and culture conditions are listed in **Table 1**. All culture media were purchased from Merck (Germany), and anaerobic condition was provided by Gas Pack (Merck).

**Preparation of the Extract**
The *C. zeylanicum* was purchased, and the plant species were approved by an expert in medicinal plants. Then, it was washed, dried, and grounded. Afterward, 100 g of the powder was added to one liter of water, boiled for 10 minutes with gentle heat, then filtrated, and spray dried.

**Determining the Minimum Inhibitory Concentration of the Extract**
*C. zeylanicum* powder was added to the broth culture medium to obtain 2, 5, 10, 20, and 50 mg/mL concentrations. Then, 10⁶ cfu/mL of each pathogen was added to each tube. The growth of the pathogen after 18 hours was detected using a spectrophotometer and compared with the control.

**Determining the Prebiotic Properties of the Extract**
*Cinnamomum zeylanicum* powder was added to the broth culture medium to obtain 2, 5, 10, 20, and 50 mg/mL concentrations. Then, 10⁶ cfu/mL of each probiotic bacteria was added to each tube. After 18 hours of incubation, probiotic growth was measured using a spectrophotometer and compared with the control.

**Statistical Analysis**
GraphPad Prism 8 (GraphPad, San Diego, CA) was used for statistical analysis. Data were presented as mean ± standard deviation. Statistically significant differences were evaluated by one-way analysis of variance, followed by Tukey’s posttest. Then, the experiments were carried out in duplicate on three different occasions, and *P*<0.05 was considered statistically significant.

**Results**

**Minimum Inhibitory Concentration of Cinnamomum zeylanicum Extract**
**Table 2** shows the minimum inhibitory concentration of *C. zeylanicum* extract.

**Determining the Prebiotic Properties of the Extract**
Figure 1 illustrates the effect of *C. zeylanicum* plant extract on the growth of *Lactobacillus reuteri* and *L. acidophilus*. *C. zeylanicum* extract exhibited prebiotic properties for both *Lactobacillus reuteri* and *L. acidophilus* bacteria.

**Discussion**

**Table 1. Bacterial Strains, Media, and Culture Condition**

<table>
<thead>
<tr>
<th>Bacterial Strains, Media, and Culture Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Species</td>
</tr>
<tr>
<td>Probiotic strains</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>Lactobacillus reuteri</td>
</tr>
<tr>
<td>Pathogens</td>
</tr>
<tr>
<td><em>Escherichia coli</em> enterohemorrhagica</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

Note. MRS: de Man, Rogosa and Sharpe; RC agar: Reinforced clostridial agar.

**Table 2. MIC of Cinnamomum zeylanicum Extract against 6 Strains of Pathogenic Bacteria**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th><em>Escherichia coli</em></th>
<th><em>Clostridium difficile</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Salmonella Typhi</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomum zeylanicum</td>
<td>MIC</td>
<td>MIC</td>
<td>MIC</td>
<td>MIC</td>
<td>MIC</td>
</tr>
<tr>
<td>Concentrations</td>
<td>1 mg/mL</td>
<td>20 mg/mL</td>
<td>5 mg/mL</td>
<td>20 mg/mL</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

Note. MIC: Minimum inhibitory concentration.

**Figure 1.** The Effect of *Cinnamomum zeylanicum* Extract on the Growth of *Lactobacillus reuteri* and *Lactobacillus acidophilus*. Note. Data are presented as mean ± standard deviation. *** Significant difference compared to control (P<0.001).
Despite the advances in medical science, 26% of deaths in developed countries are caused by infectious diseases. Although antibiotics are effective in the treatment of various infectious diseases, many microbes quickly become resistant to some of them.\textsuperscript{10,11} Medicinal plants are an important source of metabolites and compounds that can be used as antibacterial agents.\textsuperscript{10,12}

The results of the present study indicated that the extract of \textit{C. zeylanicum} has antibacterial activity against \textit{E. coli}, \textit{S. Typhi}, \textit{S. aureus}, \textit{C. difficile}, and \textit{P. aeruginosa} and positive effect on the growth of \textit{L. acidophilus} and \textit{L. reuteri}.

The antibacterial activity of \textit{C. zeylanicum} extracts has been observed in different studies, which is consistent with the results of the present study. Several studies showed that \textit{C. zeylanicum} has antibacterial activity against \textit{S. aureus}, \textit{S. Typhi}, and \textit{E. coli}.\textsuperscript{13,14}

In another study, Salma \textit{et al} evaluated the antibacterial activity of the Ethanolic extract of \textit{C. zeylanicum} bark against two food-borne pathogens: Gram-positive \textit{S. aureus} and Gram-negative \textit{E. coli}. Their results showed that ethanolic extracts are more effective against \textit{S. aureus} than against \textit{E. coli}.\textsuperscript{15}

Gajbhiye and Koyande reported growth inhibitory effects of \textit{C. zeylanicum} methanolic extract against \textit{E. coli} and \textit{S. Typhi}.\textsuperscript{16} It has been found that there are different compounds in the aqueous extract, including alkaloids, saponins, tannins, flavonoids, steroids, and terpenoids.\textsuperscript{17}

Flavonoids are low molecular weight polyphenolic compounds. Different flavonoids such as quercetin, myricitrin, murine, galangin, entadanin, rutin, and their derivatives have antibacterial activities. Studies by some researchers have demonstrated the relationship between the antimicrobial properties and liposome interactions of different flavonoids,\textsuperscript{18} and the antibacterial activity of \textit{C. zeylanicum} can be attributed to these compounds.

Another result of the study was the positive effect of \textit{C. zeylanicum} aqueous extract on the proliferation of probiotic bacteria which can be attributed to the compounds having prebiotic effects in the extract.

Nowadays, probiotics are consumed to inhibit pathogens and increase the shelf life of foods. They release antimicrobial compounds (e.g., lactic acid, bacteriocin, and hydrogen peroxide) and compete with pathogens that lead to an increase in the immune response of the host.\textsuperscript{19} Loss of normal intestinal flora as a result of taking antibiotics needs novel therapeutic methods. Prebiotics found in plants such as lactulose, fructooligosaccharides, inulin, oligofructose, galactooligosaccharides, polysaccharides, and sugar alcohols, and various types of flavonoids may lead to the proliferation of probiotic strains present in the digestive system.\textsuperscript{18,20}

It has been reported that several \textit{lactobacilli} can use the phenolic acids of the extract and grow more in the presence of the extract. Previous studies have shown that sugars, cinnamic acid, glucuronic acid, and flavonoids in the \textit{C. zeylanicum} extract can have a positive effect on the growth of lactobacillus bacteria.\textsuperscript{14}

**Conclusion**

Due to the emergence of antibiotic-resistant pathogens, plants are considered an extremely suitable alternative to eliminate more multidrug-resistant microorganisms. It can be concluded that \textit{C. zeylanicum} stem bark extract has different bioactive compounds that both have antibacterial properties and increase the growth of \textit{L. reuteri} and \textit{L. acidophilus} probiotic bacteria. Therefore, it is possible to simultaneously benefit from their antimicrobial and prebiotic effects in the treatment of infectious diseases.

**Acknowledgements**

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**Authors’ Contribution**

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Supervision: Zohreh Khodaii.
Validation: Zohreh Khodaii.
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Validation: Zohreh Khodaii.
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Writing – review & editing: Zohreh Khodaii.

**Competing Interests**

No conflict of interests is declared.

**Ethical Approval**

The research project was approved by the ethics committee of the Alborz University of Medical Sciences (ID: ir.abzums.ac.ir.rec.Alborz.rec.1393.20).

**References**


