Antibacterial Activity of Green Tea and Broccoli Extracts Against Campylobacter jejuni
Actividad Antibacteriana de Extractos de Té verde y Brócoli Frente a Campylobacter jejuni

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Abstract. Using “natural green” plant extracts or their derived products in various food and beverage applications are an increasing trend in the food industry. Organic green tea, one of the most-consumed beverages worldwide, and broccoli are rich in bioactive compounds with healthy properties. In this study, the antibacterial activity of green tea and broccoli infusion extracts, and a mix of them were examined against Campylobacter jejuni, the most important causes of bacterial foodborne illness. Green tea infusion extract, enriched in phenolic compounds, mainly in catechins, showed a strong capacity to inhibit Campylobacter growth, while broccoli extracts with lower amount of phenolic compounds did not. The results obtained in the present work suggest that green tea extract could be potentially useful to inhibit the growth of Campylobacter jejuni.

INTRODUCTION
The healthy properties of green tea (Camellia sinensis) are linked closely to the phenolic compounds present in this traditional beverage (Vinson et al., 2004). These properties regarding disease prevention have been attributed to the flavonols present in tea (mainly catechin, epicatechin, gallocatechin, epigallocatechin and epigallocatechin gallate), that are potent natural antioxidants (Tester, 2003). In the context of health-promoting foods, broccoli (Brassica oleracea) is also considered a source of bioactive phytochemicals (Moreno et al., 2006). In fact, the data available reveal broccoli to be a healthy food due to the beneficial biological effects of its phytochemicals (Boivin et al., 2009). Campylobacter species are the leading cause of bacterial foodborne diarrheal illness worldwide (Ganan et al., 2012) and today...
catechins contents than the mixed extracts, while in broccoli extracts catechins were not detected. Epigallocatechin gallate was the major catechin compound quantified in the green tea extract (Table 1). The use of broccoli with green tea added hydroxycinnamic acids and flavonols to the mixtures. In contrast, the mixed extracts infusion exhibited a reduction in the content of catechins in accordance with the reduced amount of green tea in the beverages. Antibacterial activity of the extracts against C. jejuni LP1 was qualitative evaluated. Green tea extract showed a total bactericidal effect at 2 mg/mL (Figure 1) tested after 24 h of treatment, demonstrating that the three main families of identified phenolic compounds in green tea (hydroxycinnamic acids, catechins, and flavonols) could be involved in the antibacterial activity against C. jejuni. Broccoli extract infusion enriched in hydroxycinnamic acids and flavonols (Table 1) showed a bacteriostatic effect at same concentration (2 mg/mL). Finally, mixed extract infusion consisted in green tea and broccoli (50:50) with higher amount of hydroxycinnamic acids and flavonols but lower amount of catechins than green tea showed a weak antibacterial activity (Figure 1). These results suggest that catechins present in high amounts in green tea extract exhibit potent antibacterial activity. This activity may be due to the galloyl moiety present in their structures (Shimamura et al., 2007).

**Figure 1.** Antibacterial activity of green tea, broccoli and mixed extracts infusion against C. jejuni LP1.

**ACKNOWLEDGMENTS**

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**BIBLIOGRAPHY**


**CONCLUSIONS**

In this work the green tea extract showed antibacterial activity against C. jejuni. Catechin compounds were the main responsible of the growth inhibitory effect of green tea infusion. This extract could be considered as a promising antibacterial agent potentially useful for the control of Campylobacter. Identification and quantification of the individual catechin compounds from green tea extracts could be useful to standardize the production process to obtain an enriched extract to inhibit Campylobacter growth.
is necessary to find alternative solutions to the use of antibiotics for controlling this pathogen. In this regard, there is a growing interest in the use of natural antibacterial compounds, like plant extracts rich in phenolic compounds, as food preservatives. However, there are no previous reports in the literature about the effect of green tea and broccoli extracts on C. jejuni and the phenolic compounds involved in it, in spite of its importance as a foodborne pathogen. In the present work, we report the investigation on the antibacterial activity of a green tea and broccoli extracts against C. jejuni, identifying the main phenolic compounds present in the extracts.

**MATERIALS AND METHODS**

Determination of phenolic compounds: Extracts were analyzed in a Waters HPLC system with a Luna C18 column (25 cm x 0.46 cm, 5 µm particle size; Phenomenex, UK). The mobile phase was a mixture of water/trifluoroacetic acid (99.9:0.1, v/v) (A) and acetonitrile/trifluoroacetic acid (99.9:0.1, v/v) (B). The flow rate was 1 mL/min in a linear gradient, starting with 1% B for 5 min and reaching 17% B at 15 min; this was maintained for 2 min, before reaching 25% B at 22 min, 35% B at 30 min, 50% B at 35 min and 99% B at 40 min. The monitored compounds eluted off the column in 35 min. The chromatograms of phenolic compounds were recorded at 330 nm and quantified using external standards (Sigma-Aldrich, Spain). The chromatograms of catechins were recorded and quantified at 280 nm.

Bacterial strain, growth media, and culture conditions: C. jejuni LP1, strain of clinical origin, was provided by Hospital La Paz, Madrid. Liquid growth medium for C. jejuni consisted of Brucella Broth (BB) and the agar plating medium consisted of Müeller-Hinton agar supplemented with 5% defibrinated sheep blood (MHB). Campylobacter was cultured under microaerophilic conditions (85% N2, 10% CO2, 5% O2) using a Variable Atmosphere Incubator (VAIN) (MACS-VA500) at 42°C for 48 h.

Antibacterial activity: The antibacterial activity was analyzed by a qualitative method (Silvan et al., 2013) to check the effect respect to the control growth (visual reduction of growth). The procedure was as follows: 1 mL of extracts infusion (10 mg/mL) was transferred into different flasks containing 4 mL of BB. Bacterial inocula (50 µL with 1x108 CFU/mL) were then inoculated into the flasks under aseptic conditions. All cultures were prepared in triplicate and incubated microaerobically at 42°C for 24 h (130 rpm) in the VAIN. Positive growth controls were prepared by transferring 1 mL of sterile water to 4 mL of BB and 50 µL of bacterial inocula. After incubation, 20 µL of the mixtures were plated onto fresh MHB agar and incubated microaerobically at 42°C.

**RESULTS AND DISCUSSION**

The content of total phenolic compounds in the prepared infusions was much higher in green tea (1276 ± 206 mg/100 mL) than in broccoli (34 ± 10 mg/100 mL) and mixed extracts (594 ± 43 mg/100 mL) (Table 1). When the separate classes of phenolic compounds were analyzed, catechins in green tea extract and hydroxycinnamic acids in broccoli and mixed extract were the major compounds in accordance with previous studies (Balentine et al., 2000; Jaiswal et al., 2012). Green tea infusion showed higher

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Green tea</th>
<th>Broccoli</th>
<th>Green tea 50% Broccoli 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxycinnamic acids</td>
<td>8.7 ± 2.3</td>
<td>24.7 ± 3.9</td>
<td>38.5 ± 0.9</td>
</tr>
<tr>
<td>Chlorogenic acid derivates</td>
<td>19.1 ± 0.4</td>
<td>12.0 ± 0.1</td>
<td>24.2 ± 0.2</td>
</tr>
<tr>
<td>Flavonols</td>
<td>1.5 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>2.5 ± 0.2</td>
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<tr>
<td>Quercetin derivates</td>
<td>2.3 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Catechins</td>
<td>140.6 ± 28.8</td>
<td>0</td>
<td>72.0 ± 2.4</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>52.2 ± 6.9</td>
<td>0</td>
<td>35.6 ± 2.4</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>140.9 ± 19.9</td>
<td>0</td>
<td>75.7 ± 3.1</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>911.8 ± 112.4</td>
<td>0</td>
<td>40.3 ± 0.9</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>130.20 ± 19.2</td>
<td>0</td>
<td>74.3 ± 2.3</td>
</tr>
<tr>
<td>Catechin gallate</td>
<td>518.55 ± 93.3</td>
<td>0</td>
<td>96.8 ± 11.2</td>
</tr>
<tr>
<td>Catechin gallate</td>
<td>264.89 ± 59.8</td>
<td>0</td>
<td>96.8 ± 4.9</td>
</tr>
</tbody>
</table>

Table 1. Phenolic composition (mg/100 mL) of green tea, broccoli and mixed extracts infusions.