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.***

Key words: *antibacterial agent, catechin, phenolic compounds, plant extract*

Resumen. *El uso de extractos naturales de origen vegetal o de sus productos derivados en diferentes tipos de alimentos y bebidas, presenta una creciente tendencia en la industria alimentaria. El té verde orgánico, una de las bebidas más consumidas a nivel mundial, y el brócoli, son alimentos ricos en compuestos bioactivos con propiedades saludables. En el presente estudio se examinó la potencial actividad antibacteriana de extractos de té verde y de brócoli y una mezcla de ambos frente a* ***Campylobacter jejuni****, principal patógeno bacteriano de transmisión alimentaria. Los extractos de té verde presentaron un alto contenido en compuestos fenólicos, principalmente catequinas, mostrando una gran capacidad para inhibir el crecimiento de* ***Campylobacter****, mientras que los extractos de brócoli, con un menor contenido en compuestos fenólicos, no mostraron esta capacidad. Los resultados obtenidos en este trabajo sugieren que el extracto de té verde podría ser potencialmente usado para inhibir el crecimiento de* ***Campylobacter jejuni****.*

Palabras clave: *Agente antibacteriano, catequina, compuestos fenólicos, extracto de la planta*

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 flavanols 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 no inhibitory activity 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 weakly 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).

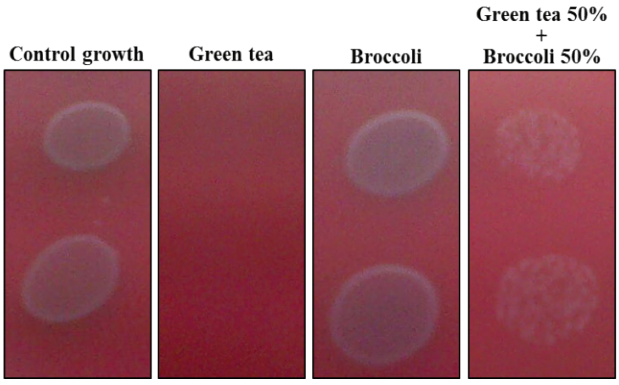

Figure 1. Antibacterial activity of green tea, broccoli and mixed extracts infusion against C. jejuni LP1.

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.

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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 42ºC 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 42ºC 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 42ºC.

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

Phenolic compounds	Green tea	Broccoli	Green tea 50% Broccoli 50%
Hydroxycinnamic acids			
Chlorogenic acid derivates	8.7 ± 2.3	24.7 ± 3.9	38.5 ± 0.9
Sinapic acid derivates	19.1 ± 0.4	12.0 ± 0.1	24.2 ± 0.2
Flavonols			
Quercetin derivates	1.5 ± 0.1	1.0 ± 0.1	2.5 ± 0.2
Kaempferol derivates	2.3 ± 0.4	1.0 ± 0.1	2.8 ± 0.2
Catechins			
Epigallocatechin	140.6 ± 28.8	0	72.0 ± 2.4
Catechin	52.2 ± 6.9	0	35.6 ± 2.4
Epicatechin	140.9 ± 19.9	0	75.7 ± 3.1
Epigallocatechin gallate	911.8 ± 112.4	0	40.3 ± 0.9
Gallocatechin gallate	130.20 ± 19.2	0	74.3 ± 2.3
Epicatechin gallate	518.55 ± 93.3	0	96.8 ± 11.2
Catechin gallate	264.89 ± 59.8	0	96.8 ± 4.9
Total phenolics	1276.7 ± 206.9	34.7 ± 10.3	594.3 ± 43.6