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Antibacterial effect of kaempferol and (−)-epicatechin on *Helicobacter pylori*

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Abstract Phenolic compounds are generated by the secondary metabolism of plants and have been associated with antibacterial properties. Among bacteria affecting human health, *Helicobacter pylori* has been associated with gastric cancer. There is limited information about the effect of individual or grouped phenolics on *H. pylori* growth. Previous studies have evaluated the effect of phenolics being part of highly complex food or beverage matrices. The aim of this work was to evaluate the in vitro antibacterial effect of kaempferol and (−)-epicatechin, both individually and combined, on *H. pylori* in liquid and solid cultures and in co-cultures with AGS human gastric carcinoma cells. Bacterial viability tests were performed in liquid cultures with subsequent CFU/mL counting and in solid cultures by measuring inhibition haloes. Kinetic curves of bacterial growth inhibition in the presence of those phenolics, and the protective effect of (−)-epicatechin on AGS cells against *H. pylori* infection were characterized. (−)-Epicatechin and kaempferol displayed antibacterial activities, being (−)-epicatechin more effective than kaempferol. After the combined application of both phenols, a synergistic effect of kaempferol plus low but not high doses of (−)-epicatechin was observed. Finally, (−)-epicatechin yielded protection to AGS cells against *H. pylori* infection.

(−)-Epicatechin and kaempferol, both individually and combined, have antibacterial properties and protective effect on *H. pylori* infection.

Keywords *Helicobacter pylori* · Antimicrobial effect · Flavonoids · Phenols

Introduction

Helicobacter pylori is a neutralophilic Gram-negative spiral-shaped bacterium that colonizes the human stomach [1]. Several studies have shown that *H. pylori* is one of the most prevalent infections throughout the world [2]. Approximately 80 % of the infected population is asymptomatic and only a small but significant fraction of the colonized patients present gastric or duodenal ulcers, mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) or gastric cancer [1–3]. *H. pylori* infection represents the major risk factor for gastric cancer which accounts annually for at least 738,000 deaths [3, 4]. It has been estimated that *H. pylori* increases ten times the probability to develop gastric cancer [5]. Eradication therapies for *H. pylori* involve the use of a proton-pump inhibitor (PPI), tetracycline, amoxicillin, clarithromycin or metronidazole, in a triple therapy [6]. However, the increased prevalence of antibiotic resistance makes *H. pylori* eradication not always successful. A recent study in Latin American countries showed that antibiotic resistance rates vary significantly by drug and by country, but not by year of sample collection [7]. However, such observation contrasts with another study from outside the Latin America region that showed rising resistance rates to certain antibiotics over time [6]. Metronidazole can reach 100 % resistance [8], whereas 30 and 20 % of all isolates are resistant to tetracycline and clarithromycin, respectively

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[9]. Considering that eradication therapies based on the use of drugs that may be unavailable in some geographic areas or that may produce undesirable secondary effects can be costly and ineffective, the search for new drugs and the development of alternative therapies has become critical [10]. Plants are attractive sources of polyphenols, a family of compounds showing promising results in the treatment of a variety of diseases. Particularly, several studies have shown that phenolic compounds found in cranberries, green tea, apple and wine, and affect *H. pylori* viability [11–19]. All those studies were based on the analysis of the effect of polyphenols from complex plant extracts. In that regard, limited information exists about the effect of pure polyphenols on *H. pylori* [20]. Kaempferol and (−)-epicatechin, two phenolic compounds, are widely distributed among plants [21, 22]. Not much is known about the effect of either pure kaempferol or pure (−)-epicatechin, or about any eventual synergy or interference between them, on *H. pylori* growth. The aim of this study was to compare the antimicrobial effect of kaempferol and (−)-epicatechin, alone or in combination, against *H. pylori* viability. Also, an eventual secondary protective effect of (−)-epicatechin on *H. pylori*-infected epithelial cells was tested.

Materials and methods

Materials

Kaempferol and (−)-epicatechin standards (Sigma-Aldrich, Santiago, Chile), cellulose acetate filters (Asahi Glass, Tokyo, Japan), bacto agar (JT Baker, Mexico), dimethyl sulfoxide (DMSO) (Winkler, Santiago, Chile), horse serum (Thermo Scientific HyClone, Utah, USA), antibiotic supplements Dent and Vitox (Oxoid, Hampshire, England) and pro-analysis grade chemicals (Merck Ltd., Santiago, Chile) were purchased.

Polyphenol solutions

Stock solutions of kaempferol and (−)-epicatechin (50 mg/mL) were prepared by dissolving each polyphenol in 100 % DMSO and 50 % methanol, respectively. Both solutions were sterilized by filtration through cellulose acetate filters (0.2 μm pore size; 25 mm diameter).

Bacterial strains and growth conditions

Two *H. pylori* strains were used in this study: ATCC 43504 (NCTC 11637), which was first isolated from the gastric antrum of an Australian patient, and ATCC 700392 (26695), which was isolated from a patient with gastritis in the UK. Frozen stocks of *H. pylori* were recovered and routinely grown for 48 h at 37 °C in 5.5 % CO2 and 70–80 % humidity on trypticase soy agar (TSA) (Becton–Dickinson, Sparks, MD 21152, USA) with 0.4 % *H. pylori* selective supplement Dent (Oxoid Basingstoke, Hampshire, England), 0.3 % Isovitalex (Oxoid) and 5 % horse serum [23–25]. For growth experiments in liquid cultures, cells were grown in trypticase soy broth (TSB) (Becton–Dickinson) with 5 % horse serum supplemented with Isovitalex and Dent. Bacteria were first grown at pH 7.0 to an optical density of 0.6–1.0 U at 600 nm (OD_{600}) and subsequently diluted to a starting OD_{600} of 0.05 U. To measure the growth of *H. pylori* in liquid medium, a serial dilution of the culture medium was prepared, aliquots of the various dilutions were plated on trypticase soy agar (TSA) plates, and the number of colonies (CFU) was determined [26].

Protective effect of phenolics on gastric cells against *H. pylori*

AGS gastric cancer cells (ATCC CRL 1739) were cultured as reported elsewhere [27]. For infection assays, AGS host cells were infected at a MOI (multiplicity of infection) of 100 (bacteria to gastric cell ratio) as described by Valenzuela et al. [27]. In a parallel assay, gastric cells were treated with (−)-epicatechin prior to infection. AGS cell viability was determined by measuring the mitochondrial reductase activity (MTT assay) according to del Campo et al. [28].

H. pylori growth assay in liquid medium

Helicobacter pylori (3×10^7 cells/mL) was inoculated in 5 mL of TSB supplemented with a range of concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) of kaempferol or (−)-epicatechin. After incubation at 37 °C for 48 h with constant shaking at 250 rpm in a controlled atmosphere incubator (5.5 % CO2 and 70 % relative humidity), bacterial growth was determined by turbidimetry at 600 nm or by counting colony forming units on TSA plates [29].

H. pylori viability assay

From each of the experimental culture tubes described in the previous section. 100 μL of aliquots was taken at the end of the incubation period to prepare serial dilutions in PBS. Aliquots of 10 μL from each of these dilutions were plated on TSA and incubated for 48 h at 37 °C [20]. The number of colony forming units per mL (CFU/mL) corresponding to each experimental condition was determined.

Inhibition kinetics of bacterial growth

The assay was conducted basically as described by Romero et al. [30] with minor modifications. Briefly, 3×10^7 CFU/

mL were inoculated into Eppendorf tubes containing 1 mL of TSB medium supplemented with 1 mg/mL of kaempferol or (−)-epicatechin and incubated at 37 °C. From this mixture, 100 μL of aliquots was removed at 0, 15, 30, 45, 60 and 90 min. The numbers of viable cells at various time points were determined as described above.

Inhibition halo test on agar plates

The procedure was performed as described by Rodríguez et al. [31]. Three-millimeter-diameter wells were made in TSA plates, and 30 μL of a series of kaempferol or (−)-epicatechin solutions (corresponding to 0–1 mg of the polyphenol) was deposited in individual wells. Then, 100 μL of *H. pylori* suspension containing 3×10^7 cells/mL was evenly spread over the plates with a metal handle loop. After 48 h of incubation at 37 °C, the diameter of each growth inhibition halo was determined.

Effect of combining kaempferol and (−)-epicatechin on *H. pylori* growth

Helicobacter pylori strains were grown on TSA plates with a series of wells containing a fixed concentration of kaempferol (0.4 mg/well) plus increasing doses of (−)-epicatechin (0.0, 0.05, 0.1, 0.2, 0.25 and 0.4 mg/well). After 48 h of incubation at 37 °C, the diameter of each growth inhibition halo was measured.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's tests were used to evaluate differences.

Results

Antibacterial activity of (−)-epicatechin against *H. pylori*

H. pylori growth in (−)-epicatechin-supplemented liquid medium

Growth of *H. pylori* strains 43504 and 26695 in the presence of (−)-epicatechin was evaluated in TBS medium supplemented with a range of (−)-epicatechin concentrations over a 48-h incubation period. After this time, CFU/mL of each cell suspension was determined. As shown in Fig. 1, the CFU/mL parameter decreased when the medium was supplemented with a range of concentrations of (−)-epicatechin. Full growth inhibition was observed with (−)-epicatechin concentrations of 0.8 and 1.0 mg/mL for *H. pylori* 43504 and 26695 strains, respectively. In this assay, the minimum
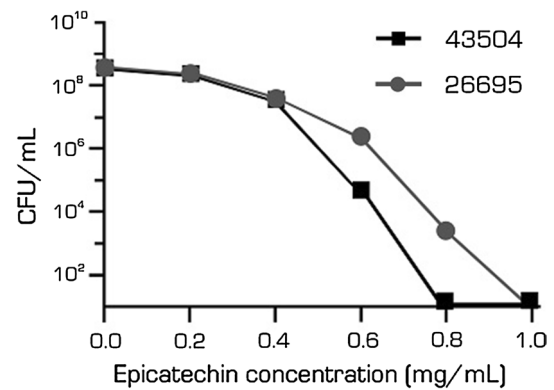
Fig. 1 Concentration-dependent bactericidal effect of (−)-epicatechin on *H. pylori* strains in liquid medium. Aliquots of culture medium taken after 48 h of culturing *H pylori* 43504 and 26695 strains in the presence of a range of (−)-epicatechin concentrations are tested by counting CFU. The figure is representative of three independent experiments. Growth inhibition in both *H. pylori* strains is significant (Tukey's test, $p \leq 0.05$) over all the range of tested (−)-epicatechin concentrations
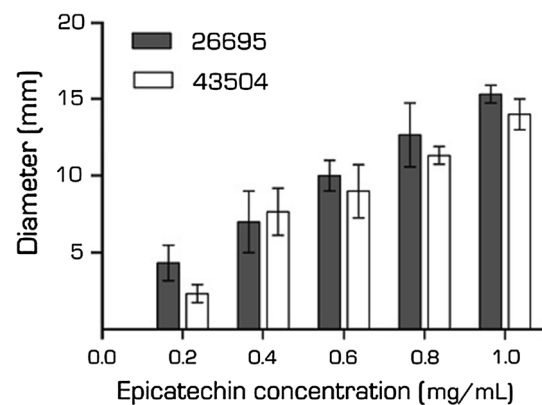

Fig. 2 Inhibitory effect of (−)-epicathechin on *H. pylori* growth in semisolid agar medium. Inhibition halo test on *H. pylori* strains exposed to (−)-epicatechin over the range 0–1 mg/well showed a progressive inhibitory effect. Values are mean ± SD ($n = 3$). No inter-strain differences are observed (Tukey's test, $p \geq 0.05$). The ordinate represents diameter of the inhibition zone around well

inhibitory concentration (MIC) of (−)-epicatechin to produce 99 % of *H. pylori* growth inhibition was 0.6 mg/mL.

Inhibition halo test for (−)-epicatechin

Bacteria were cultured for 48 h on TSA plates with a series of wells containing a range of fixed amounts of (−)-epicatechin. As shown in Fig. 2, diameters of the growth inhibition zones around the wells increased gradually with the content of (−)-epicatechin in the range from 0 mm for the control condition (only solvent) to 15 mm in the presence of 1.0 mg of (−)-epicatechin. Compared to the control, growth inhibition
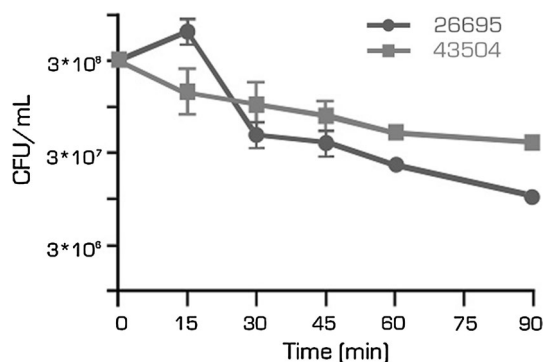
Fig. 3 Kinetics of growth inhibition of *H. pylori* by (−)-epicatechin. Liquid cultures of *H pylori* 43504 and 26695 strains are combined with 1 mg/mL (−)-epicatechin, and at the times indicated on the abscissa aliquots are taken and tested by counting CFU. Values are mean ± SD (*n* = 3). Growth inhibition in both *H. pylori* strains is directly influenced (Tukey's test, $p \leq 0.05$) by the exposure time to the polyphenol over the range tested

Fig. 4 Concentration-dependent bactericidal effect of kaempferol on *H. pylori* strains in liquid medium. The figure is representative of three independent experiments. Experimental conditions and results are identical to those described in Fig. 1 caption, except the polyphenol

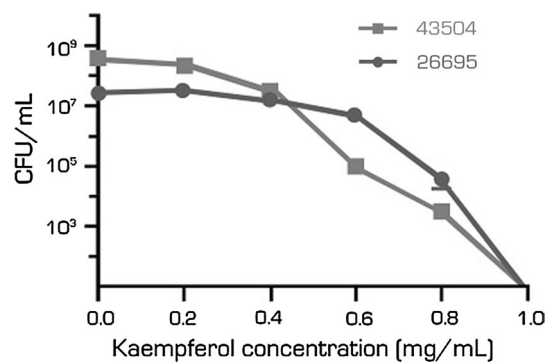halos were significantly larger ($p < 0.05$) with amounts of (−)-epicatechin equal or greater than 0.4 mg/well.

Kinetics of bacterial growth inhibition by (−)-epicatechin

Cultures of *H. pylori* 26695 and 43504 strains in TBS medium were supplemented with 1.5 mg/mL of (−)-epicatechin at time zero and at various time points; thereafter (up to 90 min), aliquots of the cell culture were seeded on TSA plates to determine the CFU/mL parameter. As shown in Fig. 3, after 90 min of exposure to (−)-epicatechin the CFU/mL values for *H. pylori* 43504 decreased from 3×10^8 at time zero to 9.5×10^7, whereas for *H. pylori* 26695 such parameter fell from 3×10^8 to 3×10^6 after the same treatment. Decreases in viabilities of both *H. pylori* strains became statistically significant ($p < 0.05$) after 60 min of incubation in the presence of (−)-epicatechin. Altogether, (−)-epicatechin provoked a dose-dependent decrease in the CFU/mL values and a dose-dependent increase in the diameters of the inhibition halos on agar plate cultures with both bacterial strains. Those effects are also consistent with the observation that the CFU/mL value decreased with increasing times of exposure of the *H. pylori* strains to such polyphenol. Accordingly, (−)-epicatechin has a significant growth-inhibitory effect and antimicrobial activity on *H. pylori*, thus having a major influence on the viability of the microorganism.

Antibacterial activity of kaempferol against *H. pylori*

H. pylori growth in kaempferol-supplemented liquid medium*

Helicobacter pylori strains 26695 and 43504 were incubated for 48 h in TBS liquid culture medium containing

a range of kaempferol concentrations (0 to 1 mg/mL) (Fig. 4). The CFU/mL values ranged from 5.2×10^8 (43504 strain) or 3.5×10^7 (26695 strain) in the control condition to 0 CFU/mL in both cultures at 1.0 mg/mL of kaempferol. Both decreases were statistically significant ($p < 0.05$).

Inhibition halo test for kaempferol

Bacteria were cultured for 48 h on TSA well plates containing a series of different amounts of kaempferol. As shown in Fig. 5, diameters of the growth inhibition halos around the wells increased gradually with the increasing content of kaempferol in the range from 0 mm for the control condition (only solvent) up to 15 mm for the presence of 1.0 mg of kaempferol. Growth inhibition halos were significantly larger than control ($p < 0.05$) with amounts of kaempferol equal or greater than 0.6 mg/well.

Kinetics of bacterial growth inhibition by kaempferol

In a similar experiment to the one described for (−)-epicatechin, the CFU/mL parameter was determined at various times after supplementation of the culture medium with 1.5 mg/mL of kaempferol. With both *H. pylori* strains, an increased viability was observed after 30 min of exposure to kaempferol. Longer times of exposure resulted in a statistically significant decrease in the CFU/mL value. Thus, after 90 min of exposure to kaempferol the CFU/mL value was reduced from 3×10^9 at time zero to 3×10^7 (Fig. 6). The inhibitory effect of kaempferol on *H. pylori* growth was found to be similar to the one observed for (−)-epicatechin. Furthermore, no statistically significant differences in the sensitivities of both *H. pylori* strains were observed. This observation suggests that the antibacterial effects of
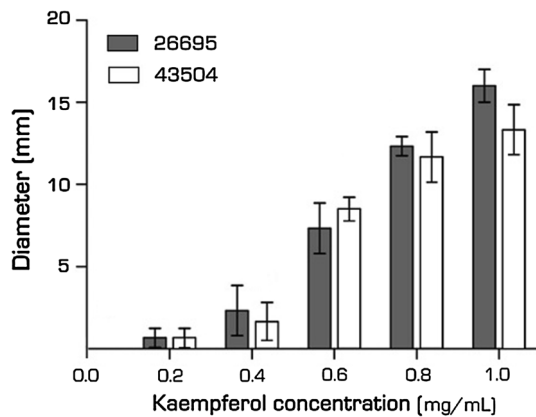
Fig. 5 Inhibitory effect of kaempferol on *H. pylori* growth in semi-solid agar medium. Experimental conditions and results are identical to those described in Fig. 2 caption, except the polyphenol. Values are mean $\pm$ SD ($n = 3$)
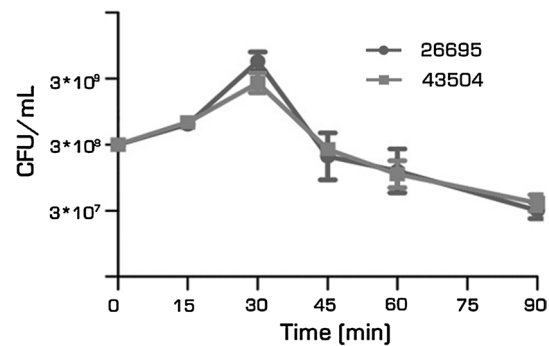

Fig. 6 Kinetics of growth inhibition of *H. pylori* by kaempferol. The study is identical to the one described under Fig. 3, excepting the polyphenol. Values are mean $\pm$ SD ($n = 3$). Although in this study growth of both *H. pylori* strains is influenced by the exposure time to the polyphenol, growth inhibition is found to be significant (Tukey's test, $p \leq 0.05$) only after at least 60 min of exposure. Both strains displayed a similar behavior

(−)-epicatechin and kaempferol are not dependent upon the bacterial strain.

Effect of combining (−)-epicatechin and kaempferol on *H. pylori* growth

Helicobacter pylori 43504 was grown on TSA well plates with a series of either a fixed concentration of (−)-epicatechin (0.25 mg/well) or kaempferol (0.5 mg/mL) plus increasing doses of kaempferol or (−)-epicatechin, respectively. After 48 h of incubation at 37 °C CFU/mL, values were determined. Table 1 summarizes a factorial arrangement of treatments with doses of 0, 50, 100 and 150 µg/mL of each compound. CFU/mL values ranged from 5.4×10^7 (control) to 1.4×10^3 (50 µg/mL (−)-epicatechin plus 150 µg/mL kaempferol). Over the whole range of tested concentrations, increasing concentrations of kaempferol in the presence of a constant dose of (−)-epicatechin resulted in further reduction in bacteria growth. A similar trend was observed with varying doses of (−)-epicatechin and a fixed amount of kaempferol. Comparatively, at similar concentrations of the polyphenol, viability of the bacterium in the presence of kaempferol was somewhat greater than that observed in the presence of (−)-epicatechin. Such effect was amplified with increasing doses of kaempferol.

(−)-Epicatechin effect on a co-culture of AGS cells and *H. pylori*

To see how *H. pylori* infection affects viability of AGS cells, cultures of these cells were infected with different *H. pylori* MOIs. After incubation for 24 h, the mitochondrial reductase activity (MTT) was determined as an index of AGS cell viability. As shown in Fig. 7a, the higher the MOI

the lower the viability of AGS cells so that at a MOI of 500 AGS cell viability was reduced to 40 %. On the other hand, viability of AGS cells in the presence of (−)-epicatechin over the range 100–500 µg/mL was unaffected or just marginally affected (Fig. 7b). To evaluate the protective effect of (−)-epicatechin on *H. pylori*-infected AGS cells, viability assays were conducted in liquid medium in the presence of either 250 or 500 µg/mL of the flavanol at a MOI of 500. As shown in Fig. 7c, viability of AGS cells decreased to 40 % at a MOI of 500 (compared to 100 % viability of uninfected AGS cells serving as control), while infection at a MOI of 500 in the presence of either 250 or 500 µg/mL (−)-epicatechin reduced AGS cell viability to 75–80 % only. These results are indicative of a protective effect of (−)-epicatechin on *H. pylori*-infected AGS cells.

Discussion

Polyphenols, a highly abundant vegetal component of the human diet, have been associated with important sensory, taxonomic, nutritional and pharmacological properties of drinks and foods [32]. Several authors have reported on the bactericidal effect of polyphenol-rich extracts against a number of microorganisms [11, 13, 16, 18, 19, 30, 33]. Such observation has stimulated research on naturally occurring substances as potential alternatives or complements for the conventional treatment of infectious diseases [12–14, 18, 19]. *H. pylori*, a bacterium that colonizes the human gastric mucosa, has been associated with various pathological conditions, including peptic ulcer, intestinal lymphoma and gastric cancer (1–3). Anti-*H. pylori* effects have been observed in polyphenol extracts from almond skins [34], muscadine grape skins [35] and blackberry

Table 1 Effect of combining (−)-epicatechin and kaempferol on *H. pylori* 43504 growth (CFU/mL)

	EC$_0$	EC$_{50}$	EC$_{100}$	EC$_{150}$
K$_0$	5383.3 ± 84.4 A a	99.45 ± 4.1 B a	88.2 ± 4.5 C a	21.69 ± 4.0 D a
K$_{50}$	73.1 ± 3.9 A b	1.48 ± 1.2 B b	4.62 ± 2.1 C b	1.28 ± 1.2 B b
K$_{100}$	53.6 ± 2.1 A b	0.89 ± 1.1 B b	0.90 ± 0.4 B c	0.89 ± 0.6 B b
K$_{150}$	28.1 ± 4.6 A b	0.14 ± 0.5 B b	0.42 ± 0.3 B c	0.37 ± 0.3 B b

K$_0$ to K$_{150}$: kaempferol 0–150 μg/mL; EC$_0$ to EC$_{150}$: epicatechin 0–150 μg/mL. Different letters (capital and single) stand for statistically significant difference (Tukey's test, $p \leq 0.05$)

Fig. 7 Protective effect of (−)-epicatechin on AGS cell viability (MTT assay) during *H. pylori* infection. **a** AGS cell survival in DMEM medium over a range of *H. pylori* MOI. **b** AGS cell survival over a range of (−)-epicatechin concentrations. **c** Survival of AGS cells is measured when their infection by *H. pylori* at a MOI 500 is produced in the presence of (−)-epicatechin at concentrations (250–500 μg/mL) having no detrimental effect on AGS viability. Under both conditions, AGS cell viability (mean ± SD, $n = 3$) is significantly higher than that observed in the absence of the polyphenol (Tukey's test, $p \leq 0.05$). EC and MOI stand for (−)-epicatechin and multiplicity of infection by *H. pylori*, respectively
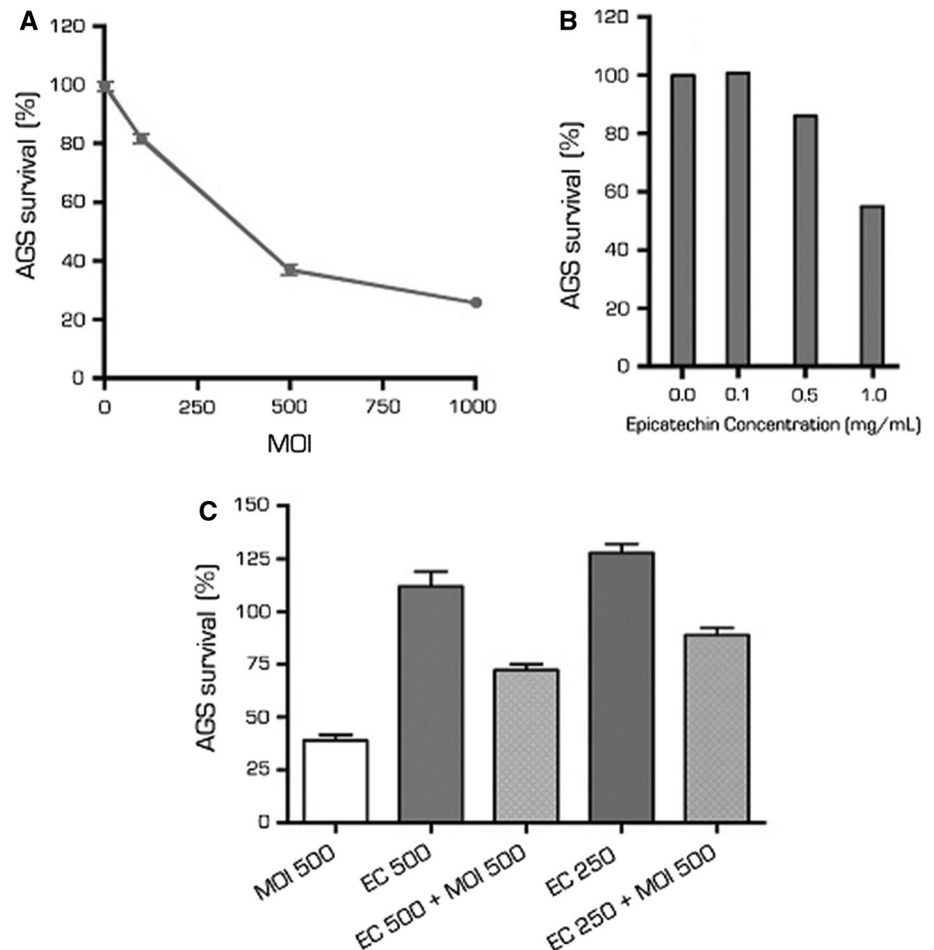
leaves [16], among others. A few studies aimed to identify particularly active anti-*H. pylori* components of those extracts have pointed recurrently to protocatechuic acid, catechin, epicatechin, ellagic acid, myricetin, quercetin, resveratrol, gallic acid, tannic acid and n-propyl gallate [20, 34, 36, 37]. In the present study, we evaluated quantitatively the individual and combined effects of kaempferol and (−)-epicatechin, two widely distributed components of plant extracts, on *H. pylori* growth in liquid and semisolid cultures. We have now shown that both polyphenols have a major growth-inhibitory effect on *H. pylori*.

Epicatechin displayed a concentration-dependent inhibitory effect on *H. pylori* growth as measured either in liquid culture medium (turbidimetry at 600 nm) or in semisolid agar plates (counting CFU and measuring growth inhibition halos). Such inhibitory effect was found to depend upon the time of exposure of the bacterium to the polyphenol. Using the same approaches, kaempferol, another member of the flavonol family of polyphenols, was also found to have growth-inhibitory effects against *H. pylori*. All those growth-inhibitory effects were identical in two widely used *H. pylori* strains (*H. pylori* 26695 and 43504). Altogether,

on a per mole basis, kaempferol and epicatechin displayed similar growth-inhibitory effects on *H. pylori*.

Considering that both epicatechin and kaempferol showed marked inhibitory effects against *H. pylori* growth and that both polyphenols are usually part of the complex composition of foods, we additionally evaluated the effect of combining epicatechin and kaempferol on the bacterium growth in liquid culture medium. Thus, mixtures of low doses of epicatechin and kaempferol displayed additive antibacterial activity. Under these experimental conditions, such additive effect was partial because the effect of both polyphenols used in combination was usually less than the sum of the individual effects. Interestingly, at higher doses of epicatechin and kaempferol neither additive nor synergistic activity was observed. These observations contradict Yanagawa et al. [17] who claim that polyphenols in plant extracts are currently less effective antibacterial compounds than the corresponding pure compounds. Anyhow, the partially additive effect of kaempferol and epicatechin on the inhibition of *H. pylori* growth needs to be further studied. In this same regard, we have recently described that other two polyphenol compounds, catechin and gallic acid, display growth-inhibitory effects in *H. pylori* and that those effects are also partially additive [20].

According to the present observations, *H. pylori* seems to display a significantly higher sensitivity (tenfold) to both epicatechin and kaempferol when compared with *E. coli*, a common gut bacteria (data not shown). Thus, these observations would lend additional support to the hypothesis that these polyphenol compounds may have differential effects on different microorganisms [20, 30]. The fact that the size of the *H. pylori* genome is a third of the one of *E. coli* may also explain on a genetic ground its lower ability to counteract the antibacterial effects of epicatechin and kaempferol and probably other polyphenols.

On the other hand, several lines of evidence indicate that *H. pylori* promotes gastric carcinogenesis depending on the direct contact of live bacteria with human cells [1–3, 27], which would affect the viability of the latter ones. Considering that (−)-epicatechin and kaempferol have been found to display significant anti-*H. pylori* effects, in this study we designed an (−)-epicatechin protection assay using AGS human gastric carcinoma cells subjected to *H. pylori* infection. As shown in this study, AGS cell viability was markedly reduced when cultured in the presence of *H. pylori*. By contrast, when the assay was carried out in the presence of 250–500 µg/mL (−)-epicatechin, AGS cell viability was just marginally affected by *H. pylori* infection, thus showing indirectly the anti-*H. pylori* effect of the polyphenol.

Altogether, the whole set of in vitro observations suggest that both kaempferol and (−)-epicatechin display growth-inhibitory effects on *H. pylori*. It remains to be clarified whether these observations have an in vivo correlate

considering that both small-sized polyphenols are efficiently absorbed by the jejunum and ileum, that they may be chemically modified in the small intestine and that they usually represent only a minor fraction of the total polyphenol content of the human diet in contrast with the abundance of non-absorbable more complex polyphenols [38, 39]. Anyhow, antibacterial activities of kaempferol and (−)-epicatechin against *H. pylori* may well become part of the growing list of beneficial effects of common polyphenol constituents of the human diet.

Conclusion

Under the conditions of this study, both (−)-epicatechin and kaempferol presented a significant inhibitory effect on *H. pylori* growth in a concentration-dependent manner. Both polyphenols affected growth of *H. pylori* 26695 and 43504 strains. At identical doses, the effect of (−)-epicatechin was higher than that of kaempferol. In liquid culture medium, at low doses both compounds showed additive antibacterial activity, while at higher doses neither additive nor synergistic activity was observed. Finally, (−)-epicatechin showed a dose-dependent protective role on AGS cell cultures against *H. pylori* infection.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

Compliance with ethics requirements The study was conducted according to the recommendations of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, University of Chile.

References

1. Dunn B, Cohen H, Blaser M (1997) *Helicobacter pylori*. Clin Microbiol Rev 10:720–741
2. Eusebi LH, Zagari RM, Bazzoli F (2014) Epidemiology of *Helicobacter pylori* infection. Helicobacter 19:1–5
3. Nagani S (2012) Carcinoma of the stomach: a review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 4:156–169
4. Parkin D (2000) Global cancer statistics in the year. Lancet Oncol 2:533–543
5. Yamaoka Y (2008) *Helicobacter pylori*—molecular genetics and cellular biology. Horizon Scientific Press, UK, p 261
6. O'Connor A, Vaira D, Gisbert JP, O'Morain C (2014) Treatment of *Helicobacter pylori* infection. Helicobacter 19:38–45
7. Camargo MC, García A, Riquelme A, Otero W, Camargo CA, Hernandez-García T, Candia R, Bruce MG, Rabkin CS (2014)

The problem of *Helicobacter pylori* resistance to antibiotics: a systematic review in Latin America. Am J Gastroenterol 109(4):485–495

8. Aboderin OA, Abdu AR, Odetoyin B, Okeke IN, Lawal OO, Ndububa DA, Agbakwuru AE, Lamikanra A (2007) Antibiotic resistance of *Helicobacter pylori* from patients in Ile-Ife, Southwest, Nigeria. Afr Health Sci 7(3):143–147

9. Vallejos C, Garrido L, Cáceres D, Madrid AM, Defilippi C, Defilippi CC, Toledo H (2007) Prevalence of metronidazole, clarithromycin and tetracycline resistance in *Helicobacter pylori* isolated from Chilean patients. Rev Med Chile 135:287–293

10. Bonacorsi C, Raddi MS, Carlos IZ, Sannomiya M, Vilegas W (2009) Anti-*Helicobacter pylori* activity and immunostimulatory effect of extracts from Byrsonima crassa Nied. (Malpighiaceae). BMC Complement Altern Med 9:2

11. Tombola F, Campello S, De Luca L, Ruggiero P, Del Giudice G, Papini E, Zoratti M (2003) Plant polyphenols inhibit VacA, a toxin secreted by the gastric pathogen *Helicobacter pylori*. FEBS Lett 543:184–189

12. Mabe K, Yamada M, Oguni I, Takahashi T (1999) *In vitro* and in vivo activities of tea catechins against *Helicobacter pylori*. Antimicrob Agents Chemother 43(7):1788–1791

13. Mahady G, Pendland S, Chadwick L (2003) Resveratrol and red wine extracts inhibit the growth of CagAþ strains of *Helicobacter pylori in vitro*. Am J Gastroenterol 98:1440–1441

14. Pastene P, Speisky H, García A, Moreno J, Troncoso M, Figueroa G (2010) In vitro and in vivo effects of apple peel polyphenols against *Helicobacter pylori*. J Agric Food Chem 58:7172–7179

15. Brown J, Huang G, Haley-Zitlin V, Jiang X (2009) Antibacterial effects of grape extracts on *Helicobacter pylori*. Appl Environ Microbiol 75:848–852

16. Martini S, D'Addario C, Colacevich A, Focardi S, Borghini F, Santucci A (2009) Figura N, Rossi C. Antimicrobial activity against *Helicobacter pylori* strains and antioxidant properties of blackberry leaves (*Rubus ulmifolius*) and isolated compounds. Int J Antimicrob Agents 34:50–59

17. Yanagawa Y, Yamamoto Y, Hara Y, Shimamura T (2003) A combination effect of epigallocatechin gallate, a major compound of green tea catechins, with antibiotics on *Helicobacter pylori* growth in vitro. Current Microbiol 47:244–249

18. Vattem D, Lin Y, Ghaedian R, Shetty K (2005) Cranberry synergies for dietary management of *Helicobacter pylori* infections. Process Biochem 40:1583–1592

19. Yahiro K, Shirasaka D, Tagashira M, Wada A, Morinaga N, Kuroda F, Choi O, Inoue M, Aoyama N, Ikeda M, Hirayama T, Moss J, Noda M (2005) Inhibitory effects of polyphenols on gastric injury by *Helicobacter pylori* VacA toxin. Helicobacter 10:231–239

20. Díaz-Gómez R, López-Solís R, Obreque-Slier E, Toledo-Araya H (2013) Comparative antibacterial effect of gallic acid and catechin against *Helicobacter pylori*. LWT Food Sci Technol 54:331–335

21. Obreque-Slier E, Peña-Neira A, López-Solís R, Zamora-Marín F, Ricardo da Silva J, Laureano O (2010) Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (*Vitis vinifera L.*) during ripening. J Agric Food Chemi 58:3591–3599

22. Monagas M, Bartolomé B, Gómez-Cordovés C (2005) Update knowledge about the presence of phenolic compounds in wine. Crit Rev Food Sci Nutr 42:85–118

23. Cerda O, Rivas A, Toledo H (2003) *Helicobacter pylori* strain ATCC 700392 encodes a methyl-accepting chemotaxis receptor protein (MCP) for arginine and sodium bicarbonate. FEMS Microbiol Letters 224:175–181

24. Toledo H, Valenzuela M, Rivas A, Jerez C (2002) Acid stress response in *Helicobacter pylori*. FEMS Microbiol Letters 213:67–72

25. Almarza O, Nuñez D, Toledo H (2014) The DNA-binding protein HU has a regulatory role in the acid stress response mechanism in *Helicobacter pylori*. Helicobacter 20:29–40

26. Toledo H, López-Solís R (2009) Tetracycline resistance in Chilean clinical isolates of *Helicobacter pylori*. J Antimicrob Chem 65:470–473

27. Valenzuela M, Perez-Perez G, Corvalán AH, Carrasco G, Urra H, Bravo D, Toledo H, Quest AFG (2010) *Helicobacter pylori* induced loss of the inhibitor-of-apoptosis protein survivin is linked to gastritis and death of human gastric cells. J Infect Dis 202:1021–1030

28. del Campo M, Toledo H, Lagos N (2013) Okadaic acid toxin at sublethal dose produced cell proliferation in gastric and colon epithelial cell lines. Mar Drugs 11:4751–4760

29. Stevens K, Sheldon B, Klapes A, Klaenhammer T (1991) Nisin treatment for inactivation of Salmonella species and other gram-negative bacteria. Appl Environ Microbiol 57:3613–3615

30. Romero C, Medina E, Vargas J, Brenes M, De Castro A (2007) In vitro activity of olive oil polyphenols against *Helicobacter pylori*. J Agric Food Chem 55:680–686

31. Rodríguez M, Alberto M, Manca de Nadra M (2007) Antibacterial effect of phenolic compounds from different wines. Food Control 18:93–101

32. Monagas M, Bartolomé B, Gómez-Cordovés C (2005) Updated knowledge about the presence of phenolic compounds in wine. Crit Rev Food Sci Nutr 45:85–118

33. Brown JC, Huang G, Haley-Zitlin V, Jiang X (2009) Antibacterial effects of grape extracts on *Helicobacter pylori*. Appl Environ Microbiol 75:848–852

34. Bisignano C, Filocamo A, La Camera E, Zummo S, Fera MT, Mandalari G (2013) Antibacterial activities of almond skins on cagA-positive and-negative clinical isolates of *Helicobacter pylori*. BMC Microbiol 13:103–109

35. Brown JC, Jiang X (2013) Activities of muscadine grape skin and polyphenolic constituents against *Helicobacter pylori*. J Appl Microbiol 114:982–991

36. Ruggiero P, Tombola F, Rossi G, Pancotto L, Lauretti L, Del Giudice G, Zoratti M (2006) Polyphenols reduce gastritis induced by *Helicobacter pylori* infection or VacA toxin administration in mice. Antimicrob Agents Chemother 50:2550–2552

37. Díaz-Gómez R, Toledo-Araya H, López-Solís R, Obreque-Slier E (2014) Combined effect of gallic acid and catechin against *Escherichia coli*. LWT Food Sci Technol 59:896–900

38. Spencer JPE (2003) Metabolism of tea flavonoids in the gastrointestinal tract. J Nutr 133(10):3255S–3261S

39. Obreque-Slier E, Peña-Neira A, López-Solís R, Zamora-Marín F, Ricardo da Silva J, Laureano JO (2010) Comparative study of the phenolic composition of seeds and skins from Carmenere and Cabernet Sauvignon grape varieties (*Vitis vinifera L.*) during ripening. J Agric Food Chem 58:3591–3599