Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2025, **16**, 5049

Investigating ellagitannins from strawberry (*Fragaria* × *ananassa* Duch.) as a new strategy to counteract *H. pylori* infection and inflammation†

Giulia Martinelli, [a] Stefano Piazza, [a] Marco Fumagalli,[a] Nicole Maranta, [a] Carola Pozzoli, [a] Elisa Sonzogni,[a] Safwa Moheb El Haddad,[a] Urska Vrhovsek,[b] Fulvio Mattivi,[b] Enrico Sangiovanni, *[a] Emma De Fabiani[a] and Mario Dell'Agli [a]

Helicobacter pylori colonises the gastric mucosa of at least 50% of the world's human population, causing a variety of gastric diseases, including chronic gastritis, peptic ulcers, and gastric cancer. The ability of the bacterium to colonise and induce inflammation is achieved through a combination of different virulence factors. Besides the conventional pharmacological treatment of *H. pylori* infection based on bacterial eradication, natural compounds could function as an adjuvant to existing therapies, potentially mitigating the consequences of *H. pylori* infection by impairing bacterial adhesion and subsequent gastric inflammation. This study focuses on *Fragaria* × *ananassa* extract and its ellagitannins, agrimoniin and casuarictin, as novel strategies to counteract *H. pylori*-related gastritis. Strawberry tannins exhibited anti-inflammatory properties in both TNFα-challenged and *H. pylori*-infected models, inhibiting the release of IL-8 and IL-6 by GES-1 cells. This effect was, at least in part, ascribed to the impairment of NF-κB signalling. The study compares the infection of epithelial cells with two different *H. pylori* strains (*cagA*+ and *cagA*–), demonstrating the different capacities of the extract and ellagitannins to impair the release of IL-6, while the effect on IL-8 release was independent of the different virulence potentials of the strains. Moreover, the extract and ellagitannins demonstrated antibacterial activity against *cagA*+ *H. pylori* growth, but exhibited reduced activity against the *cagA*– strain. The results of this study indicate the possible use of *Fragaria* × *ananassa* as a pharmacological and nutritional source in the prevention and/or treatment of gastritis induced by *H. pylori*.

Received 26th February 2025,
Accepted 15th May 2025

DOI: 10.1039/d5fo01022e

rsc.li/food-function

1. Introduction

Helicobacter pylori is a microaerophilic and Gram-negative bacterium, able to colonise the human gastric mucosa, despite the acidic environment, due to the action of various virulence factors.[1] The severity of *H. pylori* infection outcomes, including gastric ulcers and cancer, is the consequence of a complex interaction between virulence factors, bacterial strains, the host's immune response, and environmental factors.[2] Colonisation induces a pro-inflammatory response in gastric epithelial cells, leading to the recruitment of several immune cells to the submucosa at the site of infection.[3] The *cagPAI* gene, a key virulence factor, enables *H. pylor*i to attach to host cells and, through the type IV secretion system (T4SS), deliver virulent proteins like CagA into the host cytoplasm.[4] Infection activates multiple pathways (*e.g.*, NF-κB, MAPK and STAT3), leading to the production of inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNFα.[5,6] In particular, the NF-κB pathway increases the expression and release of IL-8, the main chemokine involved in gastritis.[7,8]

Current treatment of *H. pylori* infection consists of triple therapy with a proton pump inhibitor and at least two antibiotics, selected according to recent guidelines.[9] Although eradication treatment reduces gastric cancer incidence and mortality,[10] antibiotic therapy has adverse effects and disrupts the host's bacterial flora.[11] Furthermore, the increasing development of antibiotic resistance leads to treatment failure.[12] Consequently, there is an urgent need to identify innovative non-antibiotic treatments for *H. pylori* infections. Natural substances have shown considerable potential in eradicating *H. pylori* and preventing related gastric diseases,[13] such as proanthocyanidins from *Peumus boldus*[14] and EGCG.[15]

Strawberry (*Fragaria* × *ananassa* Duch.) is rich in beneficial nutrients including fibre, vitamins, minerals, and polyphe-

[a]*Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti" (DiSFeB), Università degli Studi di Milano, 20133 Milan, Italy.*
E-mail: enrico.sangiovanni@unimi.it
[b]*Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), 38098 S. Michele all'Adige, Italy*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5fo01022e

nols.[16] Among these, the tannin content, particularly the ellagitannins agrimoniin and casuarictin, has been characterised and quantified.[17] Ellagitannins are a class of hydrolysable tannins found in many seeds and fruits (*e.g.* pomegranates, berries and walnuts), are stable in the gastric environment and are metabolized by the gut microbiota to produce urolithins.[18] Ellagitannins contained in berries have exhibited anti-inflammatory[19,20] and anti-bacterial activities,[21] as reviewed by Golovinskaia *et al.* and Piazza *et al.*[22,23] Promising results have been obtained by our group investigating other sources of ellagitannins, such as chestnut leaves and the pure compound castalagin.[24] The biological activity of strawberry includes anti-cancer, anti-inflammatory, neuroprotective, cardiovascular, and antioxidant properties, in both *in vivo* and *in vitro* studies.[16,25,26]

Our previous research suggested that tannin-enriched strawberry extract possesses anti-inflammatory properties in AGS cells challenged with TNFα, by dampening the NF-κB pathway at nutritionally relevant concentrations (1–10 μg mL^{-1}). Isolated compounds revealed that casuarictin may preferentially inhibit NF-κB, while agrimoniin inhibits IL-8 secretion by also acting on other biological targets at low μM concentrations.[17] Despite promising gastric-level results, ellagitannins are still poorly investigated in *H. pylori* infection models due to the limitations of *in vitro* and *in vivo* analyses. Recently, GES-1 cells, derived from non-tumoral human epithelium, were characterized as a valuable alternative to AGS cells for studying the action of natural compounds against *H. pylori*-related gastritis.[27]

Based on these premises, the present work aims to (i) confirm the anti-inflammatory activity of strawberry extract in GES-1 cells challenged by TNFα; (ii) assess the anti-inflammatory activity of strawberry extract in *H. pylori*-infected GES-1 cells, and the antibacterial activity directly on *H. pylori*; and (iii) ascribe the biological activity to the pure ellagitannins agrimoniin and casuarictin. To gain an insight into the mode of action of strawberry tannins, the cells were infected with two different *H. pylori* strains, expressing or not the virulence factor *cagA*.

2. Materials and methods

2.1 Materials

RPMI 1640 medium, penicillin/streptomycin 100 units per mL solution, L-glutamine (2 mM), and trypsin–EDTA 0.25% solution were purchased from Gibco™ (Thermo Fisher Scientific, Rodano MI, Italy), while the Fetal Bovine Serum (FBS) and all disposable materials for cells were from Euroclone (Euroclone S.p.A., Pero, MI, Italy). The U-bottom plates were from Greiner Bio-One (Euroclone S.p.A., Pero MI, Italy). Mueller Hinton-Agar–5% sheep blood Petri dishes, defibrinated sheep blood, and Brucella broth were purchased from Thermo Fisher Diagnostics (Thermo Fisher Scientific, Rodano, MI, Italy), and the microaerophilic gas pack was from Thermo Scientific™ CampyGen™ (Thermo Fisher Scientific, Rodano MI, Italy). Lipofectamine™ 3000, CarboxyFluorescein Succinimidyl Ester (CFSE) 5 mM (CellTrace™, cell proliferation kits), and ActinRed™ 555 ReadyProbe™ Reagent were purchased from Invitrogen™ (Thermo Fisher Scientific, Rodano MI, Italy). Britelite™ Plus was from PerkinElmer (PerkinElmer, Milan, Italy). The human TNFα, human IL8 and IL6 ELISA development kits were obtained from Peprotech Inc. (Peprotech Inc., London, UK), while the human MMP9 ELISA kit was purchased from Tebu-Bio (Tebu-Bio SRL, Magenta, MI, Italy). The rabbit antibody for p-65, the secondary anti-rabbit antibody conjugated with Alexa Fluor 647, and ProLong Gold antifade reagent with 4′,6-diamidino-2-phenylindole (DAPI) (#8961) were from Cell Signaling Technology (Euroclone, S.p.A., Pero, MI, Italy). DMSO, isopropanol, glycerol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, ABTS™ solution for ELISA, and tetracycline were provided by Merck (Merck Life Science S.r.l., Milan, Italy). Epigallocatechin 3-gallate (EGCG) and apigenin were provided by Phytolab (Phytolab GmbH & Co. KG, Vestenbergsgreuth, Germany).

2.2 Strawberry extract and ellagitannin purification

Tannin-enriched extract from strawberry (*Fragaria* × *ananassa* Duch.) and purified ellagitannins were provided by The Edmund Mach Foundation (San Michele all'Adige, TN, Italy). As described in Fumagalli *et al.*,[17] strawberries were cultivated in Vigalzano (Trento, Italy) under controlled conditions to minimize the impact of environmental and agronomic factors. At the time of harvest, the fruits were healthy, and the ripening stage was evaluated as described by Gasperotti *et al.*[28] The extraction of polyphenols from the fruits was evaluated as described by the same authors,[29] and the polyphenol fraction was purified using an established method.[30] Agrimoniin and casuarictin were isolated as described by Vrhovsek *et al.*[31] and Gasperotti *et al.*,[28] respectively. The phytochemical composition of the tannin-enriched extract used in this study has been previously described.[17]

2.3 GES-1 cell culture

The GES-1 cell line (RRID: CVCL_EQ22), a non-tumoral human gastric epithelial cell line immortalized by simian virus 40 (SV-40), was kindly provided by Dr Kidane-Mulat (Howard University, College of Medicine, Washington, DC, USA). The cells were cultured in RPMI 1640 medium containing 1% penicillin/streptomycin 100 units per mL, 1% L-glutamine (2 mM), and 10% heat-inactivated FBS. Every 72 hours of incubation at 37 °C and 5% CO_2, the cells were detached from a 75 cm^2 flask using trypsin–EDTA, and 5×10^5 cells were cultured in a new flask with fresh medium.

2.4 *H. pylori* culture

The *H. pylori* 26695 *cagA*+ strain was purchased from ATCC (ATCC 700392™, Virginia, USA), while the *H. pylori cagA*− strain (named *H. pylori* #6) was obtained from Sant'Orsola Hospital (Sant'Orsola Hospital, Bologna, Italy). Both strains were characterized in terms of virulence, antibiotic resistance, and their ability to induce infection and inflammation in the

GES-1 cell line, as described in Martinelli *et al.*[27] In brief, both strains are resistant to clarithromycin and metronidazole, while they are sensitive to levofloxacin and amoxicillin. *H. pylori* 26695 *cagA*+ strain activates a broad spectrum of inflammatory genes in GES-1 cells, including the NF-κB pathway. Conversely, the pro-inflammatory effect of *H. pylori* #6 is NF-κB-independent. Both strains were cultured on agar–sheep blood Petri dishes at 37 °C and 100% humidity, under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) obtained by GasPak™ generators (BD, USA). After 72 hours, the bacteria were collected in 1× PBS using a loop and quantified by optical density (O.D.) at 600 nm. A value of O.D. = 5 corresponds to 2×10^8 bacteria. The bacteria were preserved in a medium containing 50% sheep blood, 30% Brucella broth, and 20% glycerol.

2.5 Cell treatment

Cells within 20 and 40 sub-culture passages were seeded in 24-well plates at a density of 3×10^4 cells per well and treated after 72 hours of incubation at 37 °C and under 5% CO_2. The day before the treatment, a serum starvation process was performed, using an RPMI medium with 0.5% FBS, 1% L-glutamine and 1% penicillin/streptomycin solution. Cells were challenged with TNFα (10 ng mL^{-1}) or infected with *H. pylori* (MOI of 1 : 50, cell : bacteria) for 1 or 6 hours depending on the biological assay and co-treated with strawberry extract (range: 0.1–100 μg mL^{-1}, previously dissolved in 50 : 50 H_2O : DMSO at a concentration of 50 mg mL^{-1}) and pure ellagitannins (range 0.1–50 μM, previously dissolved in DMSO at a concentration of 10 mM). Stocks of compounds under investigation were aliquoted and stored at −20 °C until the day of treatment. The treatments were conducted using serum- and antibiotic-free medium. EGCG (20 or 50 μM) and apigenin (50 μM) were used as reference anti-inflammatory compounds, as previously published.[32] The plates were maintained under an aerobic atmosphere at 37 °C and under 5% CO_2 during the treatment.

2.6 Cell viability

Cell viability was measured after 6 hours of co-treatment with the extract and ellagitannins using a 3,4,5-dimethylthiazol-2-yl-2-5-diphenyltetrazolium bromide (MTT) assay, as previously described.[24] Briefly, the medium was discarded after checking its morphological integrity using a light microscope, and 200 μL of MTT solution (0.1 mg mL^{-1} dissolved in 1× PBS) was added to each well. After incubating for 30 minutes at 37 °C in the absence of light, the MTT solution was discarded and 200 μL of isopropanol : DMSO, 90 : 10 v/v, solution was added. The absorbance was then read at 590 nm (Victor™ X3, PerkinElmer, Waltham, MA, USA). Data were calculated as % viability with respect to an unstimulated control, which was arbitrarily assigned the value of 100%. The extract and the ellagitannins, agrimoniin and casuarictin, were found to have no toxic effect on GES-1 cell viability at the concentration used (see Fig. S1 and S2†). The interference of the vehicle (DMSO) with cell viability, either human or bacterial, was excluded within the same experiments.

2.7 Measurement of NF-κB activation

NF-κB activation was measured through plasmid driven transcription after stimulation with TNFα (10 ng mL^{-1}), and through immunofluorescence techniques when cells were infected with *H. pylori* (MOI of 1 : 50, cell : bacteria). Our group previously demonstrated that the *H. pylori* 26695 *cagA*+ strain was able to induce the translocation of the p-65 subunit into cell nuclei, contrary to the *cagA*− strain.[27]

2.7.1 NF-κB driven transcription. Cells were seeded in 24-well plates at a density of 3×10^4 cells per well. The day before the treatment, they were transiently transfected with a reporter plasmid containing the luciferase gene under the control of the E-selectin promoter (NF-κB-Luc), containing κB elements responsive to NF-κB (50 ng per well). Transfection assays were performed using the Lipofectamine™ 3000 reagent, following the manufacturer's instructions. The plasmid was kindly provided by Dr N. Marx (Department of Internal Medicine-Cardiology, University of Ulm, Ulm, Germany). The day after transfection, cells were treated with TNFα and the extract or ellagitannins. After 6 hours, they were lysed using the Britelite™ Plus reagent to measure the luciferase activity, following the manufacturer's instructions as previously described.[27]

2.7.2 Immunofluorescence analysis. Cells were seeded onto coverslips in 24-well plates at a density of 1×10^4 cells per well. The day after, they were infected with the *H. pylori* 26695 *cagA*+ strain and treated with the extract or ellagitannins for 1 hour. After treatment, the cells were processed for visualization of the p-65 subunit of NF-κB inside the cell using confocal microscopy. As described in a previous work,[27] prior to treatment, *H. pylori* was stained with CFSE (5 mM) (2 μL of CFSE: 5×10^8 bacteria). The bacterial suspension was then incubated in the dark for 20 minutes at 37 °C, followed by the addition of FBS for 15 minutes at room temperature to quench the reaction. This was followed by three washes with 1× PBS and centrifugation at 3150*g* for 5 minutes to remove the excess CFSE not bound to the bacterium. After the treatment, co-cultures were rinsed with 1× PBS and fixed with a 4% formaldehyde solution for 15 minutes at room temperature. A 5% BSA blocking solution was added to the wells and incubated at room temperature for 1 hour. The cells were then incubated with a primary antibody (NF-κB p65 (D14E12) XP® Rabbit mAb #8242) diluted at 1 : 400 v/v, overnight at 4 °C. After three washes with 1× PBS, the cells were incubated with a secondary antibody (anti-rabbit IgG conjugated with Alexa Fluor 647, #4414) diluted at 1 : 1000 v/v. A 1 : 5 dilution of the ActinRed™ reagent in 1× PBS was added 30 minutes before the end of the incubation period. After 2 hours, the coverslips were washed three times with 1× PBS and mounted on slides with a decrease in DAPI (ProLong Gold Antifade Reagent with 4′,6-diamidino-2-phenylindole, #8961). The samples were then imaged using a confocal laser scanning microscope (LSM 900, Zeiss, Oberkochen, Germany).

2.8 Measurement of IL-8, IL-6 and MMP-9 release

IL-8, IL-6, and MMP-9 released in the medium were quantified by an ELISA assay after 6 hours of cell treatment with TNFα or

H. pylori strains and extract or ellagitannins, following the manufacturer's instructions as previously reported.[27] The absorbance of the samples was measured at 405 nm (Victor™ X3, PerkinElmer, Waltham, MA, USA) at the end of the assay, and compared with a standard curve made with human recombinant IL-8 (0–1000 pg mL^{-1}), IL-6 (0–1500 pg mL^{-1}), and MMP-9 (0–6000 pg mL^{-1}).

2.9 Antibacterial activity

The minimum inhibitory concentration (MIC) assay was used to measure the antibacterial activity of the extract and ellagitannins against both *H. pylori* strains, following the method previously described by Piazza *et al.*[24] Briefly, the extract and the ellagitannins were diluted in Brucella broth supplemented with 5% FBS, and then 100 µL was added to a U-bottom plate. After performing a serial dilution, 100 µL of the bacterial suspension with O.D. = 0.1 (4×10^6 cells) was added, thus obtaining a final volume of 200 µL. The plate was incubated under static conditions, for 72 hours at 37 °C under a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2), and was read at 600 nm (Victor™ X3, PerkinElmer, Waltham, MA, USA). Data were calculated as % viability with respect to the untreated bacterium, which was arbitrarily assigned the value of 100%. Tetracycline was used as a reference antibiotic compound (MIC = 0.125 µg mL^{-1}).

2.10 Statistical analysis

All biological results are expressed as the mean ± SEM of three independent experiments. Data were analysed by an unpaired ANOVA test followed by Bonferroni *post-hoc* analysis. Statistical evaluation and IC_{50} calculation were performed using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Values at $p < 0.05$ were considered statistically significant.

3. Results

3.1 Anti-inflammatory activity of *Fragaria* × *ananassa* Duch. extract and ellagitannins in GES-1 cells challenged with TNFα

Initially, we tested strawberry tannins on TNFα-treated GES-1 cells, measuring IL-8, IL-6, MMP-9 secretion and NF-κB activity to assess anti-inflammatory effects.

The strawberry extract inhibited the secretion of IL-8 and IL-6 in a concentration-dependent manner in the TNFα-treated GES-1 cells (Fig. 1A and B, respectively), with IC_{50} values of 2.09 µg mL^{-1} and 0.89 µg mL^{-1}, respectively. Furthermore, the extract inhibited the NF-κB driven transcription (Fig. 1C) with an IC_{50} value of 2.15 µg mL^{-1}, confirming the impairment of the release of these cytokines *via* the NF-κB pathway, especially for IL-8 release. The IC_{50} values for MMP-9 secretion could not be determined based on the tested extract concentrations (highest concentration tested: 5 µg mL^{-1}); however, a slight significant inhibition was observed at higher concentrations (Fig. 1D).

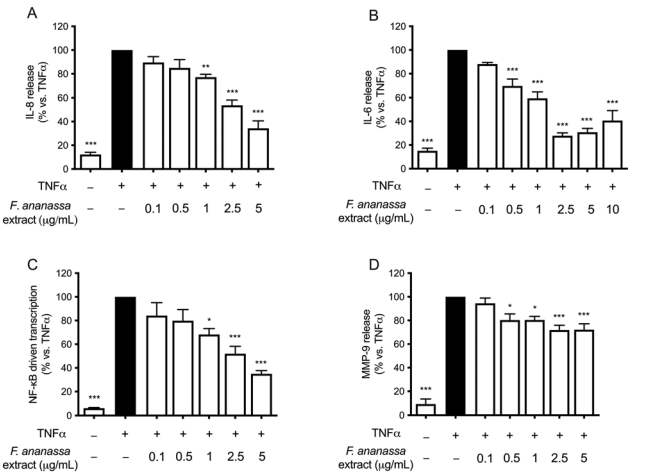

Fig. 1 The impact of the *Fragaria* × *ananassa* extract on inflammatory markers. GES-1 cells were exposed to TNFα (10 ng mL^{-1}) and the extract at different concentrations (ranging from 0.1 to 5 µg mL^{-1}) for 6 hours. The release of IL-8 (A), IL-6 (B), and MMP-9 (D) was assessed using ELISA, while the NF-κB-driven transcription (C) was measured by luciferase assay after transient transfection. The results are presented as the mean ± SEM of three experiments (n = 3) and expressed as the relative percentage compared to TNFα (black bar), which was arbitrarily assigned the value of 100%. EGCG (20 µM) was used as the reference inhibitor (medium inhibitory effect: −60%). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ *vs.* TNFα (stimulated values A: 210 ± 35 pg mL^{-1}; B: 760 ± 136 pg mL^{-1}; C: 12082 ± 3975 CPS; D: 2116 ± 336 pg mL^{-1}).

Pure ellagitannins were equally effective in inhibiting all inflammatory parameters assessed in TNFα-treated GES-1 cells. Agrimoniin and casuarictin reduced the secretion of the cytokines IL-8 and IL-6 (Fig. 2A and B, respectively), with lower IC_{50} values for the inhibition of IL-6 release, confirming the results observed in the strawberry extract. The ellagitannins inhibited NF-κB driven transcription (Fig. 2C), with IC_{50} values of 0.39 µM and 0.49 µM for agrimoniin and casuarictin, respectively. These results demonstrated that IL-8 release is mostly dependent on NF-κB activation, and both the extract and pure ellagitannins inhibited IL-8 release in an NF-κB dependent manner. The pure molecules also inhibited the MMP-9 release (Fig. 2D), with IC_{50} values of 0.50 µM and 0.47 µM for agrimoniin and casuarictin, respectively.

For all inflammatory parameters induced by TNFα in GES-1 cells, the IC_{50} values of the extract and the pure molecules are reported in Table 1.

3.2 Anti-inflammatory activity of the *Fragaria* × *ananassa* Duch. extract and ellagitannins in GES-1 cells infected with two different strains of *H. pylori*

Having observed the anti-inflammatory effects of strawberry tannins against TNFα, we tested their impact on IL-8, IL-6, and NF-κB in GES-1 cells infected with cagA+ or cagA− *H. pylori* strains.

The strawberry extract inhibited in a concentration-dependent manner the release of cytokines IL-8 and IL-6 induced by *H. pylori*-infected GES-1 cells. This effect was observed regard-

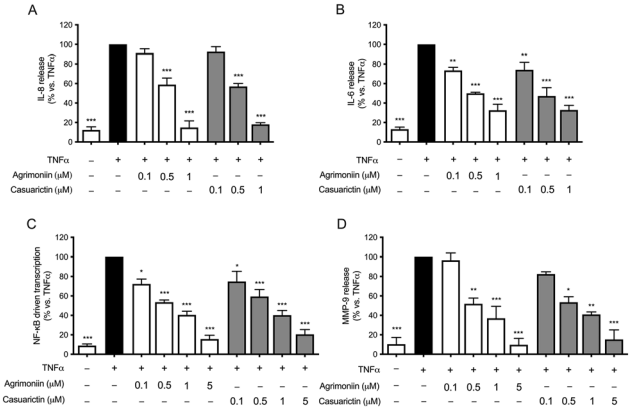

Fig. 2 The impact of ellagitannins from *Fragaria* × *ananassa* extract, agrimoniin and casuarictin on inflammatory markers. GES-1 cells were exposed to TNFα (10 ng mL^{-1}) and agrimoniin (white bars) or casuarictin (grey bars) at different concentrations (ranging from 0.1 to 5 μM) for 6 hours. The release of IL-8 (A), IL-6 (B), and MMP-9 (D) was assessed using ELISA, while the NF-κB-driven transcription (C) was measured by luciferase assay after transient transfection. The results are presented as the mean ± SEM of three experiments (n = 3) and expressed as the relative percentage compared to TNFα (black bar), which was arbitrarily assigned the value of 100%. EGCG (20 μM) was used as the reference inhibitor (medium inhibitory effect: −60%). *p < 0.05, **p < 0.01, and ***p < 0.001 *vs.* TNFα (stimulated values A: 258 ± 47 pg mL^{-1}; B: 1719 ± 770 pg mL^{-1}; C: 7442 ± 1278 CPS; D: 1973 ± 440 pg mL^{-1}).

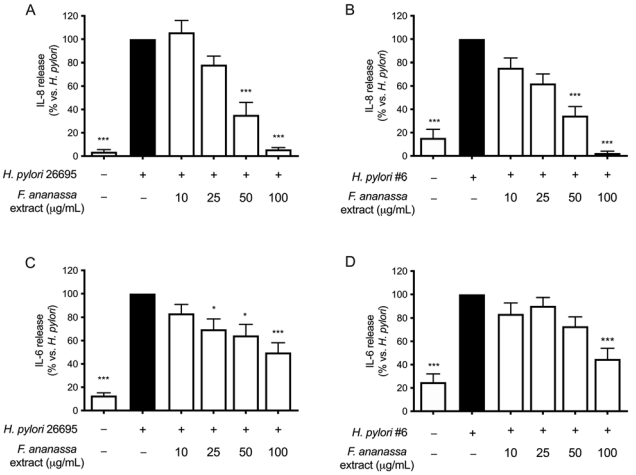

Fig. 3 The impact of *Fragaria* × *ananassa* extract on inflammatory markers. GES-1 cells were infected with *cagA*+ *H. pylori* 26695 strain or *cagA*− *H. pylori* #6 strain (MOI: 1 : 50, cell : bacteria) and the extract at different concentrations (ranging from 10 to 100 μg mL^{-1}) for 6 hours. The release of IL-8 (A and B) and IL-6 (C and D) was assessed using ELISA. The results are presented as the mean ± SEM of three experiments (n = 3) and expressed as the relative percentage compared to *H. pylori* (black bar), which was arbitrarily assigned the value of 100%. EGCG (50 μM) (inhibitory effect: −65%) and apigenin (50 μM) (inhibitory effect: −78%) were used as the reference inhibitors for IL-8 and IL-6 release, respectively. *p < 0.05 and ***p < 0.001 *vs. H. pylori* (stimulated values − A: 105 ± 44 pg mL^{-1}; B: 147 ± 53 pg mL^{-1}; C: 345 ± 90 pg mL^{-1}; D: 702 ± 184 pg mL^{-1}).

less of the bacterial strain evaluated, and the IC_{50} range of 28–68 μg mL^{-1} can be considered easily achievable *in vivo*.

The extract inhibited the release of IL-8 more effectively than IL-6 release, as evident from the IC_{50} values of 38.81 μg mL^{-1} and 28.83 μg mL^{-1} for the *cagA*+ and *cagA*− strains, respectively (Fig. 3A and B, respectively), with respect to the IC_{50} values measured for the latter, namely 68.64 μg mL^{-1} and 63.08 μg mL^{-1} for the *cagA*+ and *cagA*− strains, respectively (Fig. 3C and D, respectively). These results were in contrast to those observed before in the TNFα-treated GES-1 cells, where the impact of the extract on IL-6 was more pronounced (see Fig. 1 and Table 1). However, the results may reflect the major relevance of IL-8 impairment during *H. pylori* infection.

In our previous experiments, agrimoniin and casuarictin had similar inhibitory activities in IL-8 and IL-6 release in TNFα-treated GES-1 cells (see Fig. 2 and Table 1). In *H. pylori* infection, agrimoniin and casuarictin inhibited IL-8 release with comparable IC_{50} values. Agrimoniin showed greater activity than casuarictin, with IC_{50} values of 10.04 μM and 6.57 μM for the *cagA*+ and *cagA*− strains, respectively (Fig. 4A and B).

Casuarictin had IC_{50} values around 16 μM in both strains. Agrimoniin also inhibited IL-6 release, more effectively in the *cagA*− strain (Fig. 4C and D). Casuarictin demonstrated lower activity on IL-6, with significant inhibition only at 50 μM in *cagA*+ and inactivity in *cagA*−.

For the inflammatory parameters induced by *H. pylori* strains in GES-1 cells, the IC_{50} values of the extract and the

Table 1 Summary of IC_{50} values of strawberry (*Fragaria* × *ananassa*) extract and ellagitannins, agrimoniin and casuarictin, under TNFα-induced inflammation in GES-1 cells

	IL-8 release		IL-6 release		MMP-9 release		NF-κB driven transcription	
	IC_{50} (μg mL^{-1})	CI (95%)	IC_{50} (μg mL^{-1})	CI (95%)	IC_{50} (μg mL^{-1})	CI (95%)	IC_{50} (μg mL^{-1})	CI (95%)
Strawberry extract	2.09	1.67 to 2.63	0.89	0.60 to 1.32	>5[a]	—	2.15	1.31 to 3.51
	IC_{50} (μM)	CI (95%)	IC_{50} (μM)	CI (95%)	IC_{50} (μM)	CI (95%)	IC_{50} (μM)	CI (95%)
Agrimoniin	0.51	0.45 to 0.58	0.28	0.19 to 0.42	0.50	0.25 to 0.87	0.39	0.27 to 0.55
Casuarictin	0.50	0.44 to 0.57	0.28	0.16 to 0.48	0.47	0.25 to 0.87	0.49	0.28 to 0.83

IC_{50}: half-maximal inhibitory concentration, CI (95%): confidence interval 95%. [a] 5 μg mL^{-1} is the maximum concentration tested.

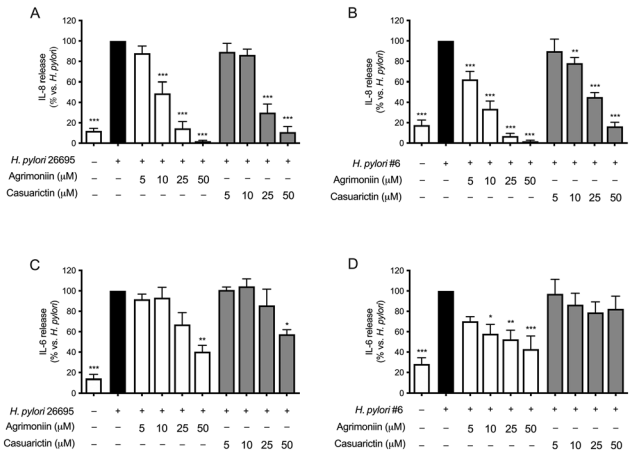

Fig. 4 The impact of ellagitannins from *Fragaria* × *ananassa* extract, agrimoniin and casuarictin on inflammatory markers. GES-1 cells were infected with *cagA*+ *H. pylori* 26695 strain or *cagA*− *H. pylori* #6 strain (MOI 1 : 50, cell : bacteria) and agrimoniin (white bars) or casuarictin (grey bars) at different concentrations (ranging from 5 to 50 μM) for 6 hours. The release of IL-8 (A and B) and IL-6 (C and D) was assessed using ELISA. The results are presented as the mean ± SEM of three experiments (n = 3) and expressed as the relative percentage compared to *H. pylori* (black bar), which was arbitrarily assigned the value of 100%. EGCG (50 μM) (inhibitory effect: −65%) and apigenin (50 μM) (inhibitory effect: −78%) were used as the reference inhibitors for IL-8 and IL-6 release, respectively. *p < 0.05, **p < 0.01, and ***p < 0.001 *vs. H. pylori* (stimulated values − A: 366 ± 63 pg mL^{-1}; B: 222 ± 41 pg mL^{-1}; C: 784 ± 33 pg mL^{-1}; D: 413 ± 74 pg mL^{-1}).

pure molecules are reported in Table 2. To gain further insight into the mechanisms of action of strawberry tannins during *cagA*+ *H. pylori* infection, it was hypothesized that they might impair the NF-κB pathway. In this study, only the *cagA*+ *H. pylori* strain was used to investigate the activation and translocation of the p-65 subunit inside the GES-1 cell nuclei.

The strawberry extract at 100 μg mL^{-1}, the highest concentration that inhibits IL-8 release, was investigated for NF-κB pathway impairment. Agrimoniin and casuarictin at 5 μM, reflecting their proportion in the extract, were also tested. As shown in Fig. 5, these treatments inhibited the translocation of the p-65 subunit into the nuclei of *H. pylori*-infected GES-1 cells, corroborating the involvement of the NF-κB pathway in the anti-inflammatory activities.

3.3 Anti-bacterial activity of *Fragaria* × *ananassa* Duch. extract and pure ellagitannins, agrimoniin and casuarictin

In human gastric epithelial cells, strawberry extract and ellagitannins inhibited selected inflammatory mediators induced by *H. pylori* infection. The anti-inflammatory activity varied depending on the bacterial virulence, as shown in Table 2. We investigated whether the strawberry extract and pure ellagitannins could inhibit the growth of the two *H. pylori* strains and whether their antibacterial activity was affected by virulence factors.

Our experiments confirm the antibacterial activities of strawberry and ellagitannins against our *cagA*+ *H. pylori* strain. The extract showed a MIC of 125 μg mL^{-1}, while agrimoniin and casuarictin had MICs of 25 μM and 50 μM, respectively (Fig. 6A and C). Against the *cagA*− strain, the extract and agrimoniin were only active at the highest concentrations tested (500 μg mL^{-1} and 100 μM), whereas casuarictin showed no activity (Fig. 6B and D). This indicates that the antibacterial activity may be influenced by bacterial virulence.

4. Discussion

Fragaria × *ananassa* is one of the most consumed fruits in Europe; it has been studied for health benefits and preventive effects on inflammation, oxidative stress, cardiovascular disease, obesity, type 2 diabetes, and neurodegenerative diseases.[16] Beyond vitamins and minerals, strawberries are rich in polyphenols, including flavonoids, phenolic acids, and tannins.[16] The tannin content, particularly the ellagitannins agrimoniin and casuarictin, has been characterised.[17,28,31] A tannin-enriched strawberry extract, with anthocyanins removed, containing 4.29% agrimoniin and 4.65% casuarictin, was previously shown to counteract inflammation in AGS cells

Table 2 Summary of the IC_{50} values of the *Fragaria* × *ananassa* extract and the ellagitannins, agrimoniin and casuarictin, on *H. pylori*-induced inflammation in GES-1 cells

	H. pylori strain	IL-8 release		IL-6 release	
		IC_{50} (μg mL^{-1})	CI (95%)	IC_{50} (μg mL^{-1})	CI (95%)
Strawberry extract	*cagA*+ 26 695	38.81	31.55 to 47.74	68.64	31.62 to 149.0
	cagA− #6	28.83	22.01 to 37.76	63.08	44.45 to 89.52
		IC_{50} (μM)	CI (95%)	IC_{50} (μM)	CI (95%)
Agrimoniin	*cagA*+ 26 695	10.04	8.15 to 12.37	32.36	21.88 to 47.87
	cagA− #6	6.57	5.42 to 7.97	7.22	1.92 to 27.08
Casuarictin	*cagA*+ 26 695	16.64	13.43 to 20.62	49.77	35.08 to 70.60
	cagA− #6	16.3	12.87 to 20.63	Not active[a]	Not active[a]

IC_{50}: half-maximal inhibitory concentration, CI (95%): confidence interval 95%. [a] 50 μM is the maximum concentration tested.

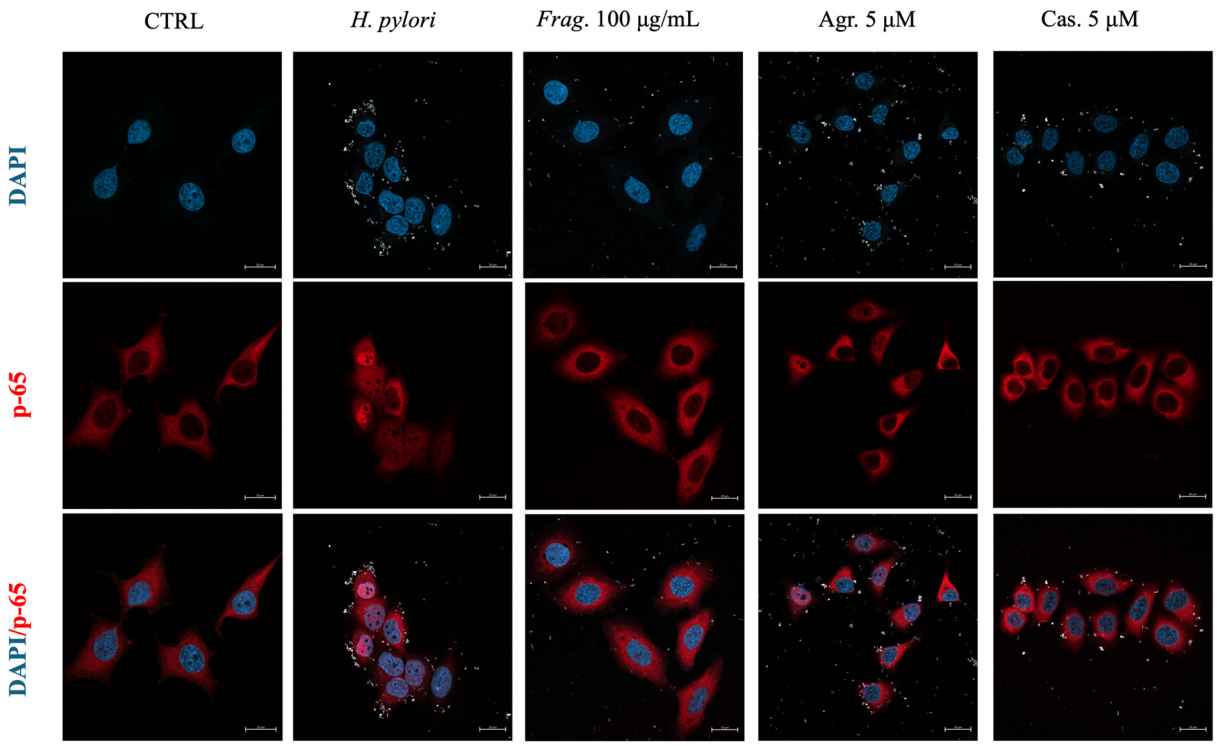

Fig. 5 The impact of *Fragaria* × *ananassa* extract and ellagitannins, agrimoniin and casuarictin, on the NF-κB translocation. GES-1 cells were infected with *H. pylori* 26695 (MOI 1 : 50, cell : bacteria) and treated with extract or pure compounds at selected concentrations (100 µg mL^{-1} and 5 µM) for 1 hour. The translocation of the p65 subunit of NF-κB (red) induced by *H. pylori* (white) into the nuclei of cells (blue) was visualized by confocal microscopy using immunofluorescence techniques (magnification: ×60, 50 µm). *Frag.*, *Fragaria* × *ananassa* extract; Agr., agrimoniin; Cas., casuarictin.

challenged with TNFα, by dampening the NF-κB pathway at nutritionally relevant concentrations (1–10 µg mL^{-1}).[17] Recently, GES-1 cells, a non-tumoral gastric epithelial model, were characterized and compared to the AGS cell line.[27] The present study employs this model to study the anti-inflammatory effects of a tannin-enriched strawberry extract and pure ellagitannins. The extract demonstrated a concentration-dependent inhibitory effect on the secretion of IL-8 and IL-6 in TNFα-treated GES-1 cells, with IC_{50} values of approximately 2.0 µg mL^{-1} and 0.90 µg mL^{-1}, respectively. Furthermore, the NF-κB-driven transcription was inhibited with IC_{50} values comparable to those found active on IL-8 secretion, indicating that the mechanism of action may be ascribed to the impairment of this pathway. In contrast, the secretion of IL-6 was inhibited at a lower concentration, potentially due to the involvement of additional pathways beyond NF-κB-mediated IL-6 secretion (Table 1 and Fig. 1). Similarly, agrimoniin and casuarictin exhibited comparable inhibition patterns on IL-8, MMP-9, and NF-κB-driven transcription (IC_{50} values around 0.5 µM), and inhibited IL-6 secretion at lower concentrations (Table 1 and Fig. 2).

We then employed the same model (GES-1) infected with two distinct strains of *H. pylori*, one *cagA*+ and one *cagA*−, to assess the anti-inflammatory and anti-bacterial activities of strawberry tannins. The *cagA* gene is a bacterial virulence factor that plays a pivotal role in the development of inflammation, and the subsequent probability of gastric cancer.[4,12] The strawberry extract demonstrated the capacity to inhibit the release of cytokines IL-8 and IL-6 by *H. pylori*-infected GES-1 cells in a concentration-dependent manner, regardless of strain virulence (the IC_{50} values are indicated in Table 2). It also inhibited the NF-κB pathway at 100 µg mL^{-1}, corroborating results with TNFα challenge. However, agrimoniin and casuarictin exhibited different activities depending on the strain. Both inhibited IL-8 release in both strains (IC_{50} value: 10–15 µM) and NF-κB activation induced by *cagA*+ *H. pylori*[27] at 5 µM, which reflects the percentage of pure molecules present in the strawberry extract. This suggests that ellagitannins may partly account for pathway inhibition (Fig. 5). Agrimoniin was the only compound inhibiting IL-6 release in both strains. Casuarictin slightly inhibited IL-6 secretion in *cagA*+ infection, but was inactive in *cagA*− infection (Fig. 4), indicating that it may not affect inflammation activated by *cagA*− bacteria.

Overall, in TNFα-stimulated GES-1 cells, which predominantly activate NF-κB, both ellagitannins exhibit similar anti-inflammatory profiles. However, in *H. pylori* infection, which triggers a more complex response, agrimoniin displays a more comprehensive profile by inhibiting inflammation independently of *cagA* presence.

Previous studies have demonstrated the anti-bacterial activity of certain berries, including strawberries, and certain

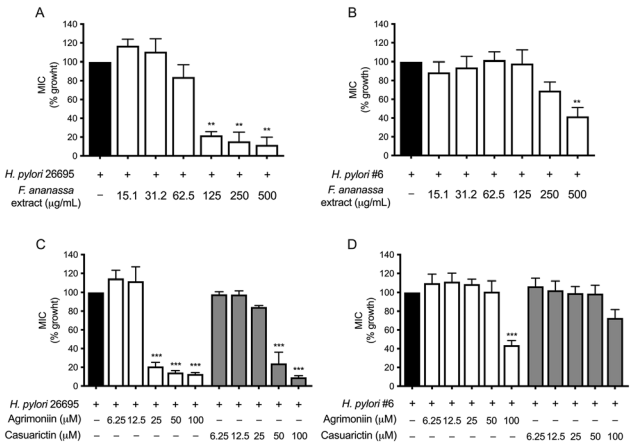

Fig. 6 The impact of the *Fragaria* × *ananassa* extract and ellagitannins, agrimoniin and casuarictin, on the *H. pylori* growth. *cagA*+ *H. pylori* 26695 strain and *cagA*− *H. pylori* #6 strain were grown with the extract (ranging from 15.1 to 500 μg mL^{-1}) or pure compounds (ranging from 6.25 to 100 μM) for 72 hours. The antibacterial activity of the extract (A and B) and pure compounds (C and D) was calculated using a MIC assay. The results are presented as the mean ± SEM of three experiments (n = 3) and expressed as the relative percentage compared to *H. pylori* (black bar), which was arbitrarily assigned the value of 100%. Tetracycline (0.125 μg mL^{-1}) was used as the reference antibiotic. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs. H. pylori*.

ellagitannins against *H. pylori*, with MIC values ranging from 6.5 μM to 50 μM, but using different strains than ours.[21,33] We confirmed antibacterial activity against the *cagA*+ strain by the strawberry extract (MIC: 125 μg mL^{-1}) and ellagitannins (MICs: 25 μM for agrimoniin and 50 μM for casuarictin). Against the *cagA*− strain, the extract and agrimoniin were active only at the highest concentration tested; casuarictin was ineffective (Fig. 6).

5. Conclusions

In conclusion, these results suggest that the antibacterial activity of strawberry extract and ellagitannins may depend on *H. pylori* strain virulence. They may be employed in addition to eradication therapy to control virulent *H. pylori* strains and the related inflammation, at concentrations achievable in the stomach.

This work reports, for the first time, an evaluation of the anti-inflammatory activity of *Fragaria* × *ananassa* in the context of two different *H. pylori* infections. Furthermore, the results confirm the extract's antibacterial activity, more pronounced against the virulent bacterial strain (*cagA*+). Thus, strawberries may be considered a potential natural product to complement eradication therapy, controlling bacterial infection and inflammation at the nutritional and pharmacological levels. The activities observed can be partly attributed to the ellagitannins agrimoniin and casuarictin. Future studies will focus on evaluating the effects of strawberry extract and its key ellagitannins in more complex systems, such as gastric organoids and *in vivo* models of *H. pylori* infection. These approaches will help to better understand their potential translational application in gastrointestinal health.

Abbreviations

AGS	Human gastric adenocarcinoma epithelial cell line
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
cagA	Cytotoxin-associated gene A
cagPAI	Cytotoxin-associated gene pathogenicity island
CFSE	Carboxy fluorescein succinimidyl ester
DAPI	4′,6-Diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
EGCG	Epigallocatechin-3-gallate
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
GES-1	Non-tumoral human gastric epithelial cell line immortalized by SV-40
H. pylori	*Helicobacter pylori*
IC_{50}	Half maximal inhibitory concentration
IL	Interleukin
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
MAPK	Mitogen-activated protein kinase
MIC	Minimum inhibitory concentration
MMP-9	Matrix metalloproteinase-9
MOI	Multiplicity of infection
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
O.D.	Optical density
RPMI	Roswell Park Memorial Institute (medium)
SEM	Standard error of the mean
STAT3	Signal transducer and activator of transcription 3
SV-40	Simian virus 40
T4SS	Type IV secretion system
TNFα	Tumor necrosis factor alpha

Author contributions

G. M.: conceptualization, investigation, formal analysis, and writing – original draft preparation; S. P.: investigation, formal analysis, data curation, and writing – review & editing; M. F.: data curation and methodology; N. M.: investigation and formal analysis; C. P.: investigation; E. S. O.: investigation; S. M. E. H.: investigation; U. V.: investigation and methodology; F. M.: investigation and methodology; E. S. A.: conceptualization, supervision, and writing – review & editing; E. D. F.: conceptualization and writing – review & editing; and M. D. A.: conceptualization, supervision, writing – original draft preparation, funding acquisition, and project administration.

Data availability

The data supporting this article have been included as part of the paper and/or the ESI.†

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors thank Dr Dawit Kidane-Mulat (Howard University, College of Medicine, Washington, DC, USA) for providing the GES-1 cells. This research was supported by grants from MIUR "Progetto Eccellenza" 2023-2027.

References

1 S. Suerbaum and P. Michetti, *Helicobacter pylori* infection, *N. Engl. J. Med.*, 2002, **347**, 1175–1186.
2 K. Robinson, R. Kenefeck, E. L. Pidgeon, S. Shakib, S. Patel, R. J. Polson, A. M. Zaitoun and J. C. Atherton, *Helicobacter pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses, *Gut*, 2008, **57**, 1375–1385.
3 K. W. Cook, D. P. Letley, R. J. Ingram, E. Staples, H. Skjoldmose, J. C. Atherton and K. Robinson, CCL20/CCR6-mediated migration of regulatory T cells to the *Helicobacter pylori*-infected human gastric mucosa, *Gut*, 2014, **63**, 1550–1559.
4 S. Backert, R. Haas, M. Gerhard and M. Naumann, The *Helicobacter pylori* type IV secretion system encoded by the cag pathogenicity island: Architecture, function, and signaling, *Curr. Top. Microbiol. Immunol.*, 2017, **413**, 187–220.
5 S. Z. Ding, J. B. Goldberg and M. Hatakeyama, *Helicobacter pylori* infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis, *Future Oncol.*, 2010, **6**, 851–862.
6 A. Lamb and L. F. Chen, Role of the *Helicobacter pylori*-induced inflammatory response in the development of gastric cancer, *J. Cell. Biochem.*, 2013, **114**, 491–497.
7 J. E. Crabtree, J. I. Wyatt, L. K. Trejdosiewicz, P. Peichl, P. H. Nichols, N. Ramsay, J. N. Primrose and I. J. Lindley, Interleukin-8 expression in *Helicobacter pylori* infected, normal, and neoplastic gastroduodenal mucosa, *J. Clin. Pathol.*, 1994, **47**, 61–66.
8 S. A. Sharma, M. K. Tummuru, M. J. Blaser and L. D. Kerr, Activation of IL-8 gene expression by *Helicobacter pylori* is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells, *J. Immunol.*, 1998, **160**, 2401–2407.
9 C. A. Fallone, S. F. Moss and P. Malfertheiner, Reconciliation of recent *Helicobacter pylori* treatment guidelines in a time of increasing resistance to antibiotics, *Gastroenterology*, 2019, **157**(1), 44–53.
10 A. C. Ford, Y. Yuan and P. Moayyedi, *Helicobacter pylori* eradication therapy to prevent gastric cancer: Systematic review and meta-analysis, *Gut*, 2020, **69**(12), 2113–2121.
11 J. M. Liou, X. T. Jiang, C. C. Chen, J. C. Luo, M. J. Bair, P. Y. Chen, C. K. Chou, Y. J. Fang, M. J. Chen, C. C. Chen, J. Y. Lee, T. H. Yang, C. C. Yu, C. C. Kuo, M. C. Chiu, C. Y. Chen, C. T. Shun, W. H. Hu, M. H. Tsai, Y. C. Hsu, C. H. Tseng, C. Y. Chang, J. T. Lin, E. M. El-Omar and M. S. Wu, Taiwan Gastrointestinal Disease and Helicobacter Consortium, Second-line levofloxacin-based quadruple therapy versus bismuth-based quadruple therapy for *Helicobacter pylori* eradication and long-term changes to the gut microbiota and antibiotic resistome: A multicentre, open-label, randomised controlled trial., *Lancet Gastroenterol. Hepatol.*, 2023, **8**(3), 228–241.
12 P. Malfertheiner, M. C. Camargo, E. El-Omar, J. M. Liou, R. Peek, C. Schulz, S. I. Smith and S. Suerbaum, *Helicobacter pylori* infection, *Nat. Rev. Dis. Primers*, 2023, **9**(1), 19.
13 C. Sousa, R. Ferreira, N. F. Azevedo, M. Oleastro, J. Azeredo, C. Figueiredo and L. D. R. Melo, *Helicobacter pylori* infection: From standard to alternative treatment strategies., *Crit. Rev. Microbiol.*, 2022, **48**(3), 376–396.
14 E. Pastene, V. Parada, M. Avello, A. Ruiz and A. Garcia, Catechin-based procyanidins from *Peumus boldus* Mol. aqueous extract inhibit *Helicobacter pylori* urease and adherence to adenocarcinoma gastric cells, *Phytother. Res.*, 2014, **28**, 1637–1645.
15 K. M. Lee, M. Yeo, J. S. Choue, J. H. Jin, S. J. Park, J. Y. Cheong, K. J. Lee, J. H. Kim and K. B. Hahm, Protective mechanism of epigallocatechin-3-gallate against *Helicobacter pylori*-induced gastric epithelial cytotoxicity via the blockage of TLR-4 signaling, *Helicobacter*, 2004, **9**, 632–642.
16 F. Giampieri, S. Tulipani, J. M. Alvarez-Suarez, J. L. Quiles, B. Mezzetti and M. Battino, The strawberry: Composition, nutritional quality, and impact on human health, *Nutrition*, 2012, **28**, 9–19.
17 M. Fumagalli, E. Sangiovanni, U. Vrhovsek, S. Piazza, E. Colombo, M. Gasperotti, F. Mattivi, E. De Fabiani and M. Dell'Agli, Strawberry tannins inhibit IL-8 secretion in a cell model of gastric inflammation., *Pharmacol. Res.*, 2016, **111**, 703–712.
18 F. A. Tomás-Barberán, A. González-Sarrías, R. García-Villalba, M. A. Núñez-Sánchez, M. V. Selma, M. T. García-Conesa and J. C. Espín, Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status, *Mol. Nutr. Food Res.*, 2017, **61**(1), DOI: **10.1002/mnfr.201500901**.
19 D. M. Grochowski, K. Skalicka-Woźniak, I. E. Orhan, J. Xiao, M. Locatelli, J. P. Piwowarski, S. Granica and M. Tomczyk, A comprehensive review of agrimoniin, *Ann. N. Y. Acad. Sci.*, 2017, **1401**, 166–180.
20 E. Sangiovanni, U. Vrhovsek, G. Rossoni, E. Colombo, C. Brunelli, L. Brembati, S. Trivulzio, M. Gasperotti,

F. Mattivi, E. Bosisio and M. Dell'Agli, Ellagitannins from *Rubus* berries for the control of gastric inflammation: In vitro and in vivo studies, *PLoS One*, 2013, **8**, e71762.

21 K. Funatogawa, S. Hayashi, H. Shimomura, T. Yoshida, T. Hatano, H. Ito and Y. Hirai, Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*, *Microbiol. Immunol.*, 2004, **48**(4), 251–261.

22 O. Golovinskaia and C. K. Wang, Review of functional and pharmacological activities of berries, *Molecules*, 2021, **26**(13), 3904.

23 S. Piazza, M. Fumagalli, S. Khalilpour, G. Martinelli, A. Magnavacca, M. Dell'Agli and E. Sangiovanni, A review of the potential benefits of plants producing berries in skin disorders, *Antioxidants*, 2020, **9**(6), 542.

24 S. Piazza, G. Martinelli, M. Fumagalli, C. Pozzoli, N. Maranta, F. Giavarini, L. Colombo, G. Nicotra, S. F. Vicentini, F. Genova, E. De Fabiani, E. Sangiovanni and M. Dell'Agli, Ellagitannins from *Castanea sativa* Mill. leaf extracts impair *H. pylori* viability and infection-induced inflammation in human gastric epithelial cells, *Nutrients*, 2023, **15**(6), 1504.

25 J. M. Alvarez-Suarez, D. Dekanski, S. Ristić, N. V. Radonjić, N. D. Petronijević, F. Giampieri, P. Astolfi, A. M. González-Paramás, C. Santos-Buelga, S. Tulipani, J. L. Quiles, B. Mezzetti and M. Battino, Strawberry polyphenols attenuate ethanol-induced gastric lesions in rats by activation of antioxidant enzymes and attenuation of MDA increase, *PLoS One*, 2011, **6**, e25878.

26 F. Giampieri, T. Y. Forbes-Hernandez, M. Gasparrini, S. Afrin, D. Cianciosi, P. Reboredo-Rodriguez, A. Varela-Lopez, J. L. Quiles, B. Mezzetti and M. Battino, The healthy effects of strawberry bioactive compounds on molecular pathways related to chronic diseases, *Ann. N. Y. Acad. Sci.*, 2017, **1398**, 62–71.

27 G. Martinelli, M. Fumagalli, S. Piazza, N. Maranta, F. Genova, P. Sperandeo, E. Sangiovanni, A. Polissi, M. Dell'Agli and E. De Fabiani, Investigating the molecular mechanisms underlying early response to inflammation and *Helicobacter pylori* infection in human gastric epithelial cells, *Int. J. Mol. Sci.*, 2023, **24**(20), 15147.

28 M. Gasperotti, D. Masuero, G. Guella, L. Palmieri, P. Martinatti, E. Pojer, F. Mattivi and U. Vrhovsek, Evolution of ellagitannin content and profile during fruit ripening in *Fragaria* spp, *J. Agric. Food Chem.*, 2013, **61**, 8597–8607.

29 M. Gasperotti, D. Masuero, F. Mattivi and U. Vrhovsek, Overall dietary polyphenol intake in a bowl of strawberries: The influence of *Fragaria* spp in nutritional studies, *J. Funct. Foods*, 2015, **18**, 1057–1069.

30 M. Gasperotti, D. Masuero, U. Vrhovsek, G. Guella and F. Mattivi, Profiling and accurate quantification of *Rubus* ellagitannins and ellagic acid conjugates using direct UPLC-Q-TOF HDMS and HPLC-DAD analysis, *J. Agric. Food Chem.*, 2010, **58**(8), 4602–4616.

31 U. Vrhovsek, G. Guella, M. Gasperotti, E. Pojer, M. Zancato and F. Mattivi, Clarifying the identity of the main ellagitannin in the fruit of the strawberry, *Fragaria vesca* and *Fragaria ananassa* Duch, *J. Agric. Food Chem.*, 2012, **60**, 2507–2516.

32 M. Bottoni, G. Martinelli, N. Maranta, E. Sabato, F. Milani, L. Colombo, P. S. Colombo, S. Piazza, E. Sangiovanni, C. Giuliani, P. Bruschi, G. Vistoli, M. Dell'Agli and G. Fico, From primary data to ethnopharmacological investigations on *Achillea erba-rotta* subsp. moschata (Wulfen) I. Richardson as a remedy against gastric ailments in Valmalenco (Italy), *Plants*, 2024, **13**(4), 539.

33 A. Chatterjee, T. Yasmin, D. Bagchi and S. J. Stohs, Inhibition of *Helicobacter pylori* in vitro by various berry extracts, with enhanced susceptibility to clarithromycin, *Mol. Cell. Biochem.*, 2004, **265**(1–2), 19–26.

