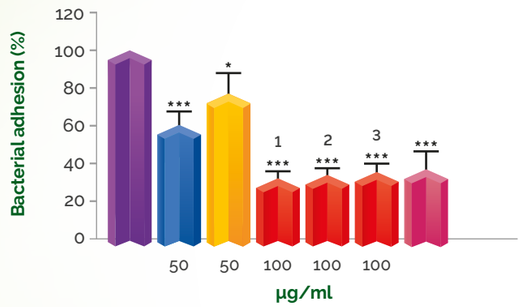
Rev. 00/2024

ANTI-ADHESIVE ACTIVITY

H. Pylori infects gastric mucosa by attaching to epithelial cells, beneath the protective mucus layer. *Cistus x incanus L.* (CI) had shown anti-adhesive properties in a previous investigation by cytofluorimetric analysis; CI impaired the adhesion of *H.pylori* to gastric cells.
To address the stability of polyphenols, **gastric digestion** was simulated in vitro and the **effects on the adhesion of *H. pylori* to GES-1 cells** were evaluated **using digested extracts** (*Cistus* × *incanus* L. = CI dig. and *Castanea sativa* Mill. = CS dig.).
GES-1 were infected with *H. pylori* and treated with CI dig., CS dig. or their combination (Mix, 100 µg/mL) for 1 hour during infection. **Procyanidin A2** was used as a **reference inhibitor.**
Bacterial adhesion was measured by FACS analysis. **CI dig.** showed a **significative anti-adhesive effect,** similar to that of the reference inhibitor, which is **maintained in all the combinations**, proving an additional effect between the extracts also after simulated gastric digestion.



LEGEND

- *H. pylori* -CSFE (50:1)
- (CI) *Cistus x incanus* L. dig. (50 µg/mL)
- (CS) *Castanea sativa* Mill. dig. (50 µg/mL)
- Mix 1, combination of CI/CS dig. 75:25
  Mix 2, combination of CI/CS dig. 50:50
  Mix 3, combination of CI/CS dig. 25:75
- PA (Procyanidin A2), 500 µM

Anti-adhesive activity of Gastalagin and its extracts
*p <0.05, ***p<0.001 vs H. pylori; ## p<0.01 vs CS dig. MFI, median fluorescence intensity

The polyphenolic composition and biological activity of *Cistus* × *incanus* L. and *Castanea sativa* Mill. extracts are only partially overlapped: the antibacterial and antiadhesive effects are prevalent for *Cistus* while the anti-inflammatory effect prevails in chestnut leaves. Moreover, biological properties of *Castanea sativa* Mill. leaf extracts seem to be related to ellagitannins, which are negligible in *Cistus* × *incanus* L., supporting the importance of the phytocomplex and a **plausible advantage in using the blend Gastalagin in respect to the use of each extract alone**.

REFERENCES: 1. Piazza, S.; Martinelli, G.; Fumagalli, M.; Pozzoli, C.; Maranta, N.; Giavarini, F.; Colombo, L.; Nicotra, G.; Vicentini, S.F.; Genova, F.; et al. Ellagitannins from *Castanea sativa* Mill. Leaf Extracts Impair H. pylori Viability and Infection-Induced Inflammation in Human Gastric Epithelial Cells. Nutrients 2023, 15, 1504. https://doi.org/10.3390/nu15061504.
2. Martinelli, G.; Fumagalli, M.; Pozzoli, C.; Nicotra, G.; Vicentini, S.F.; Maranta, N.; Sangiovanni, E.; Dell'Agli, M.; Piazza, S. Exploring In Vitro the Combination of *Cistus* × *incanus* L. and *Castanea sativa* Mill. Extracts as Food Supplement Ingredients against H. pylori Infection. Foods 2024, 13, 40. https://doi.org/10.3390/foods1301004

DNA® CERTIFIED EXTRACTS

Gastalagin®

High quality dry extract standardized in polyphenols (castalagin and vescalagin)

FOR GASTRIC DISCOMFORT

Gastalagin® is a blend of *Castanea sativa* Mill. and *Cistus x incanus* L. with anti-inflammatory activity on the gastric mucosa and with a specific antibacterial action on *H. pylori* (patent N. IT102021000023282)

Castanea sativa Mill., the chestnut tree, is a deciduous species native to the Northern Emisphere

Chestnut leaves mainly come from Italian supply chains, in the frame of an important requalification program of Lombardy chestnut forests.

Cistus x incanus, Pink Rockrose, is an evergreen, fragrant shrub, distributed along the coasts of the Mediterranean area.

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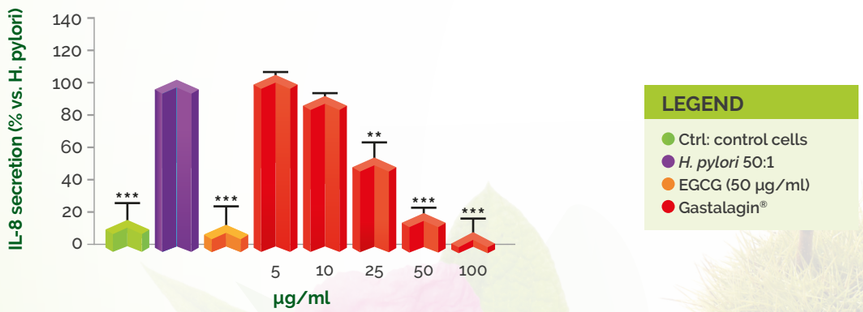
Gastritis is an inflammatory pathology mainly caused by *Helicobacter pylori*, a gram-negative bacterium commonly found in the stomach: according to literature data, the infection affects approximately 50% of the world's population.

High levels of polyphenols were found in hydroalcoholic extracts from both chestnut leaves (*Castanea sativa* L.) and *Cistus x incanus* herb. Among polyphenols, the ellagitannin isomers castalagin and vescalagin were identified as potential bioactive compounds.

ANTI-INFLAMMATORY ACTIVITY OF THE BLEND

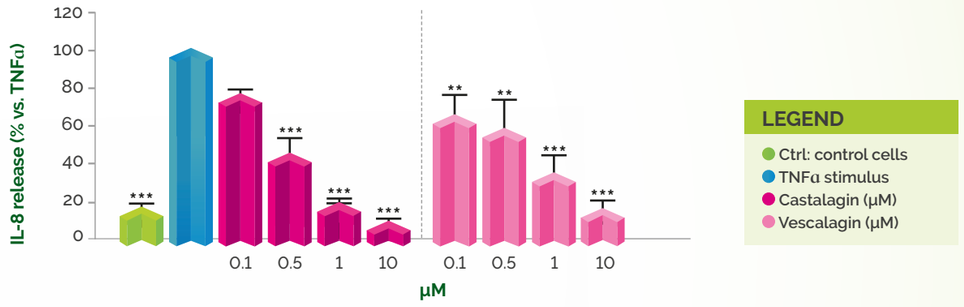
In vitro studies demonstrated that Gastalagin® inhibits the release of IL-8 in GES-1 cells (normal human gastric epithelial cell line) previously infected by *H. pylori*; the effect is mediated by NF-kB, a nuclear transcription factor, that plays a key role in regulating the response to infection.



Effect of Gastalagin® on the release of IL-8. ** $p < 0.01$; *** $p < 0.001$. IC_{50}: 24.41 µg/ml.

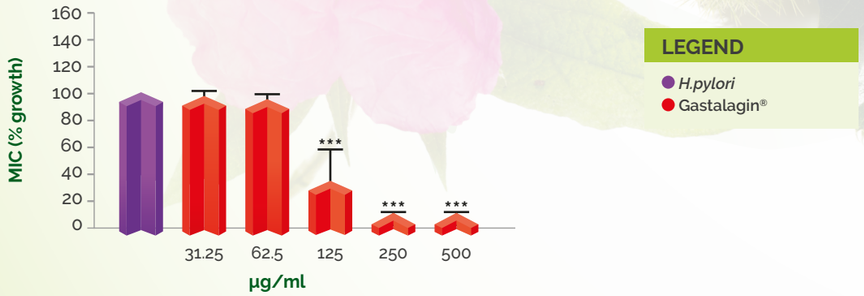
ANTI-INFLAMMATORY ACTIVITY OF CASTALAGIN AND VESCALAGIN

Ellagitannins from *Castanea sativa* Mill. (castalagin or vescalagin) strongly inhibit IL-8 release in GES-1 cells, previously treated for 6 h with TNFα (10 ng/mL). IL-8 was measured by ELISA assay.



Effect of castalagin and vescalagin on TNFα-induced IL-8 release. ** $p < 0.01$; and*** $p < 0.001$. vs. stimulus

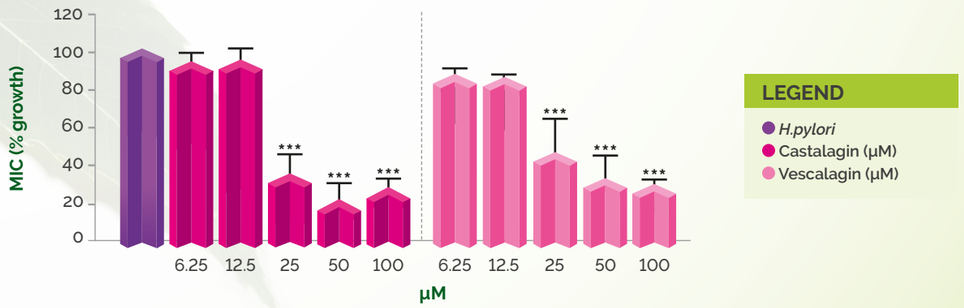
ANTIBACTERIAL ACTIVITY OF THE BLEND



Antibacterial effect of Gastalagin®. *** $p < 0.001$. MIC 125 µg/ml

ANTIBACTERIAL ACTIVITY OF CASTALAGIN AND VESCALAGIN

Castalagin and vescalagin had never been investigated for their antibacterial properties against *H. pylori* before; *H. pylori* was treated for 72h with ellagitannins: both ellagitannins caused a significant decrease in bacterial growth, with MIC between 25 and 100 µM
The rate of bacterial growth was measured as optical density (600 nm) using a photometer.



Antibacterial effect of castalagin and vescalagin. *** $p < 0.001$ vs. *H. pylori*