Potential therapeutic use of olive leaf extracts obtained from the olive tree (*Olea europaea*) against *Helicobacter pylori* infection

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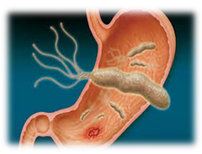
INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the major human pathogens infecting approximately 50% of the world's population [1]. *H. pylori* could be implicated in the pathogenesis of gastritis, peptic ulcer disease, gastric carcinoma, and gastric lymphoma. Its treatment is based on the combined use of different antibiotics, however, in last years, the number of antibiotic resistant strains have been increased [3]. Therefore, new alternative therapies to antibiotic are required for *H. pylori* treatment. Moreover, there is increasing evidence that *H. pylori* infection can induce oxidative stress in host cells and an inflammatory process that conditions an immunological response both local and systemic. These events may represent an important mechanism leading to epithelial injury in *H. pylori* infection. The aim of the present work was to evaluate the antibacterial, anti-inflammatory and antioxidant effect of two olive leaf extracts (OLE) against antibiotics resistant *H. pylori* strains.

MATERIALS & METHODS

- **Bacterial strains, growth media and culture conditions**
  - ✓ Seven *H. pylori* strains isolated from gastric mucosal biopsy from symptomatic patients were used in the present study.
  - ✓ Selective growth media: Brucella Broth (BB) and Mueller-Hinton agar supplemented with 5% sheep blood (MHB).
  - ✓ Growth conditions: strains reactivation into MHB and incubation for 72h at 37°C, microaerophilic atmosphere in VAIN workstation (85% N_2, 10% CO_2, 5% O_2).



Helicobacter pylori

VAIN workstation

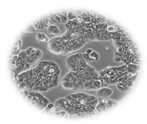
- **Olive leaf extracts (OLEs)**
  - ✓ OLE1: enriched in hydroxytyrosol (10%)
  - ✓ OLE2: enriched in oleuropein (20%)

- **Antibacterial activity**
  - ✓ Assay:
    - 1 mL of extracts disolved in BB (or only BB for control growth)
    - 4 mL BB
    - 100 µL bacterial inoculum (~1x10^8 CFU/mL)

    } Bacterial culture

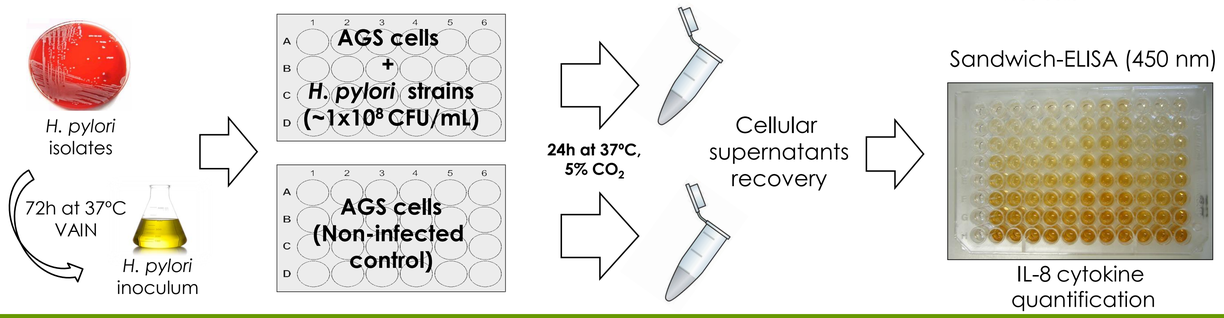
    - Incubation for 48 h at 37°C, 150 rpm, in microaerophilic atmosphere (VAIN)
    - Serial decimal dilutions of mixtures were plated onto fresh MHB agar
    - Incubation microaerobically for 72h at 37°C microaerophilic atmosphere (VAIN)
    - Antibacterial activity and MCI determination by CFU counting

- **Cell culture conditions**
  - ✓ Human gastric epithelial AGS cell line were used.
  - ✓ Seed cells in 24-well plates (~5x10^5 cells/well), incubation for 24h at 37°C, 5% CO_2
- **Antioxidant activity against intracellular reactive oxygen species (ROS)**
  - ✓ AGS cells were pre-incubated with olive leaf extracts (1 mg/mL) 2h, 37°C, 5% CO_2
  - ✓ Wash cells with PBS and incubation with fluorescent probe (DCFDA) 30 min
  - ✓ Wash cells with PBS and infection with *H. pylori* strains for 3h
  - ✓ Fluorescence measure: λ_{exc}= 485 nm λ_{em}= 530 nm



AGS cells

- **Anti-inflammatory response on infected-AGS cells**



RESULTS

Antibacterial activity against *H. pylori* strains

Table 1. Antibacterial activity of OLEs on the viable counts of different *H. pylori* strains and MIC values after 48h of treatment at 2 mg/mL. Results are expressed as log CFU/mL ± standard deviation (n = 3).

Strains	Bacterial control growth	OLE1 (10% Hydroxytyrosol)			OLE2 (20% Oleuropein)		
		2 mg/mL	log CFU/mL reduction	MIC (mg/mL)	2 mg/mL	log CFU/mL reduction	MIC (mg/mL)
Hp44	7.82 ± 0.03	< 1.5*	7.82	1.0	< 1.5*	7.82	2.0
Hp48	7.69 ± 0.02	< 1.5*	7.69	1.5	5.55 ± 0.13	2.14	2.0
Hp53	7.48 ± 0.02	< 1.5*	7.48	0.5	6.83 ± 0.01	0.65	-
Hp58	6.69 ± 0.33	< 1.5*	6.69	1.5	< 1.5*	6.69	2.0
Hp59	7.74 ± 0.01	< 1.5*	7.74	2.0	5.95 ± 0.03	1.79	2.0
Hp61	7.56 ± 0.03	< 1.5*	7.56	2.0	7.51	0.05	-
Hp19449	6.22 ± 0.09	< 1.5*	6.22	0.5	< 1.5*	6.22	0.5

MIC: minimal inhibitory concentration.
* Colony forming unit (CFU), detection limit was 1.5 log CFU/mL (30 CFU per plate).

Antioxidant activity against intracellular reactive oxygen species (ROS) production on *H. pylori* infected-AGS cells

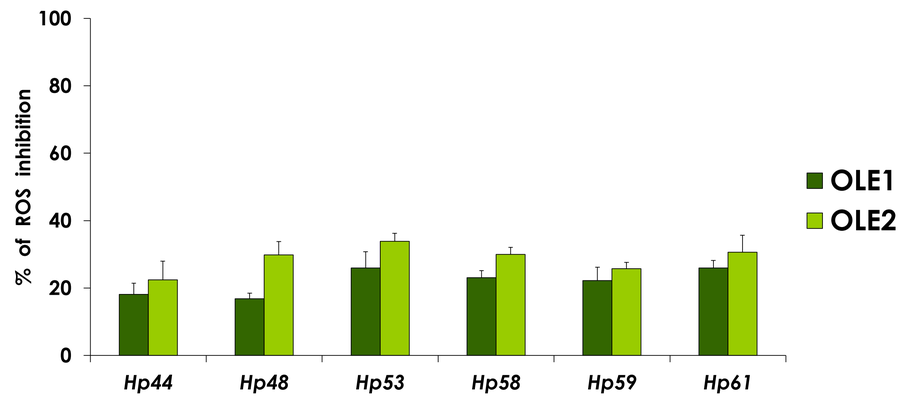


Figure 1. Protective effect of olive leaf extracts (1 mg/mL) on intracellular ROS production.

Anti-inflammatory activity on *H. pylori* infected-AGS cells

Table 2. Effect of OLEs (1 mg/mL) on IL-8 production in AGS cells infected by *H. pylori* strains. Values of IL-8 production are expressed as pg/mL (mean ± standard deviation) (n = 3).

Strains	Control	OLE1	OLE2
AGS cells	225.0 ± 21.2	-	-
Hp44	1119.4 ± 112.3	318.8 ± 23.0 (71.5%)*	212.5 ± 5.3 (81.0%)*
Hp48	3116.3 ± 49.5	2510.0 ± 350.0 (19.5%)*	158.1 ± 6.2 (94.9%)*
Hp53	2413.8 ± 30.1	618.1 ± 69.8 (74.4%)*	225.0 ± 76.0 (90.7%)*
Hp58	733.1 ± 6.2	421.9 ± 39.8 (42.5%)*	166.3 ± 5.3 (77.3%)*
Hp59	2825.6 ± 94.6	1911.9 ± 9.7 (32.3%)*	187.5 ± 24.7 (93.4%)*
Hp61	955.0 ± 24.7	374.4 ± 110.5 (60.8%)*	271.9 ± 8.0 (71.5%)*
Hp19449	449.4 ± 46.8	201.9 ± 45.1 (55.1%)*	189.4 ± 1.0 (57.9%)*

()* % of inhibition of Il-8 production respect to the control group.

CONCLUSIONS

- ❑ OLE1 completely inhibited the bacterial growth of all tested strains following a 48h exposure to 2 mg/mL, suggesting a broad antibacterial activity. Minimal inhibitory concentration (MIC) of OLE1 ranged from 0.5 to 2 mg/mL.
- ❑ OLE2 showed bactericidal activity (after 48h at 2 mg/mL) against three of the seven (3/7) tested strains (*Hp44*, *Hp58*, and *Hp19449*), and reduced 1 and 2 log CFU the bacterial growth of *Hp59* and *Hp48* strains, respectively, compared with controls growth.
- ❑ Both extracts reduced up to 33% the production of intracellular reactive oxygen species (ROS) in human gastric AGS cells infected by *H. pylori*, being the antioxidant activity of the OLE2 extract higher than OLE1in all cases.
- ❑ All *H. pylori* strains induced IL-8 production from AGS cells in a strain-dependent manner.
- ❑ OLE1 and OLE2 showed anti-inflammatory activity, reducing IL-8 pro-inflammatory factor secretion by infected-AGS cells in a range around of 20-74% and 71-93%, respectively.
- ❑ Therefore, the olive leaf extracts could be consider as a potential new candidate for *H. pylori* treatment, providing an alternative for the 20% of infected people with symptoms for whom antibiotic treatments are not effective. Furthermore, the recycling of olive industry by-products could also contribute to its revalorization, reducing also the environmental impact.

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