In vitro antibacterial activity against *Helicobacter pylori* of oligomeric and highly polymerised procyanidin-rich fractions from grape seed extract

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Introduction

Helicobacter pylori (*H. pylori*) affects approximately 50% of the world´s population, sometimes causing chronic active gastritis, which can progress to peptic ulcer and gastric cancer. Resistance to antibiotics is increasing and people demand new natural antimicrobials effective against *H. pylori*, also being an option for the 20% of patients with symptoms for whom antibiotic treatment is ineffective.

Grape seed extracts (GSE) are among the most studied plant-derived products known for their high antibacterial activities. Some publications show that GSE can contribute to the inhibition of the growth of relevant human pathogens such as *H. pylori* [1,2]. They relate this activity with their procyanidin content and, particularly, with the oligomeric procyanidin (OPC) fraction.

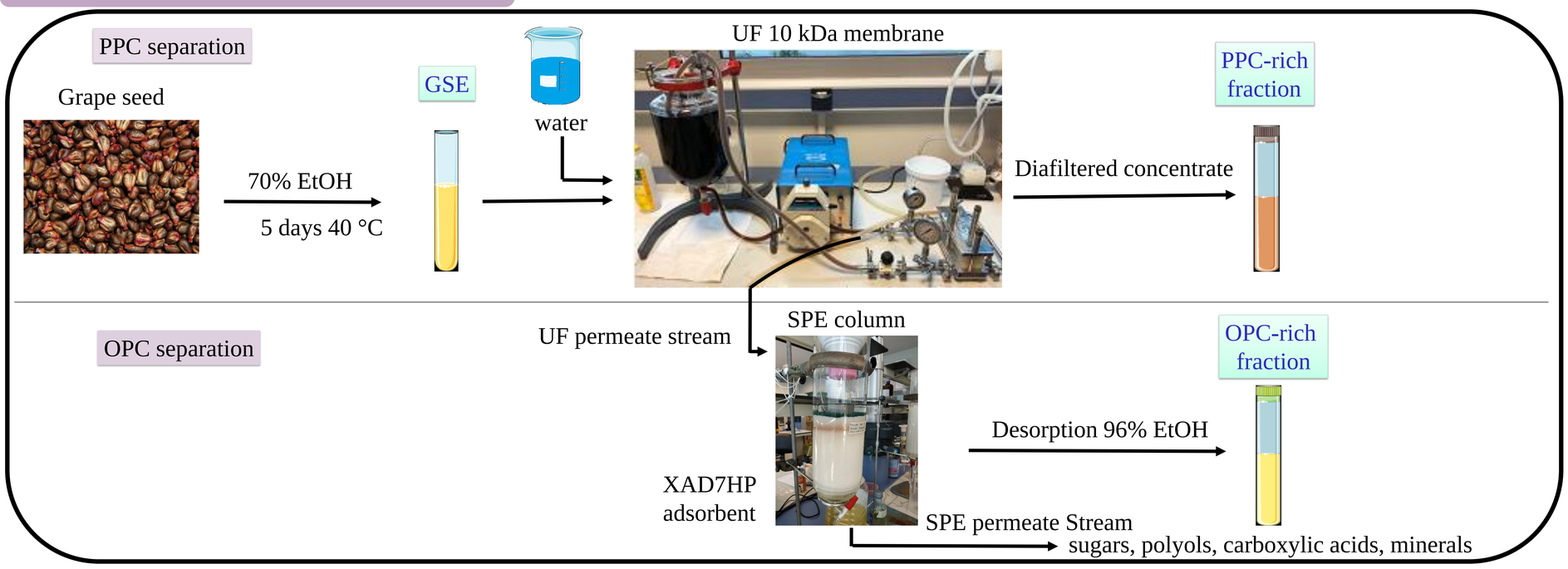
Objective

The aim of this study was to evaluate the antibacterial activity against *H. pylori* of two procyanidin fractions, one enriched in OPC and another, enriched in polymeric procyanidins (PPC), both obtained from a GSE by preparative ultrafiltration and solid-phase extraction processes.

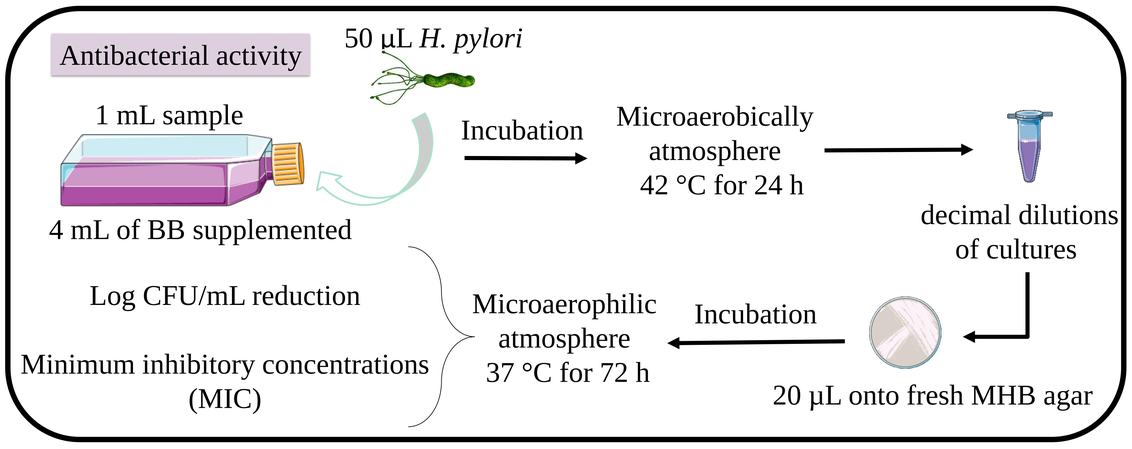
Conclusions

The whole GSE and the OPC-rich and PPC-rich fractions had high antibacterial activity against *H. pylori*. Nevertheless, the PPC-rich fraction had the highest activity against *H. pylori*, due to the highest content of total procyanidins of this fraction.

Materials and methods



Chemical Characterization	Method
Total phenolic (TPh)	Folin-Ciocalteu assay
Total procyanidin (TPC)	Acid butanol assay
Total carbohydrate (TCH)	GC-FID-MS with previous derivatization by oximes
Total catechins, total OPC and total PPC	NP-HPLC-PAD



Results and Discussion

Table 1 Total phenolic, total procyanidin, and total carbohydrate contents of GSE, PPC-rich and OPC-rich fractions, expressed in g/100 g of dry matter.

Analytical parameters	GSE	PPC	OPC
(TPh)	25.1 ± 0.5	34.9 ± 0.5	49.5 ± 1.3
(TPC)	8.5 ± 0.3	14.6 ± 0.5	12.9 ± 0.4
(TCH)	10.5 ± 0.2	3.6 ± 0.2	0.36 ± 0.02

1. The TPh indicates that PPC and OPC fractions contained almost 1.4 and 2-fold higher amounts of phenolic compounds than the GSE.

2. The TPC showed that higher enrichment of procianydins was achieved in the PPC-rich fraction than the OPC-rich fraction, but in both cases, it was lower than 2-folds.

3. GSE showed higher amounts of sugars, therefore, it should be considered that the value of total phenols in the GSE could be somewhat overestimate.

4. Results from NP-HPLC show that major components of the GSE were PPC (84%). Separation by UF allowed to obtain a PPC-rich fraction up to 96% of the total flavan-3-ol content. Purification by SPE allowed the recovery of OPC-rich fraction up to 58% of its total flavan-3-ol content. Nevertheless, 42% of PPC remained present in this fraction.

Table 2 Effects of GSE, OPC-rich and PPC-rich fractions at 2 mg/mL on the viable counts of different *H. pylori* strains. Results are expressed as log CFU/mL ± SD (n = 3).

Strains	GSE		PPC		OPC	
	log CFU/mL reduction	MIC (mg/mL)	log CFU/mL reduction	MIC (mg/mL)	log CFU/mL reduction	MIC (mg/mL)
Hp44	2.87	0.075	2.19	0.075	1.88	0.25
Hp48	5.79	0.5	3.07	0.05	1.64	0.25
Hp53	5.28	0.075	3.56	0.05	2.60	0.05
Hp58	4.04	1.5	4.89	0.05	3.49	0.1
Hp59	3.79	0.5	4.35	0.1	1.24	0.5
Hp61	3.24	0.075	4.29	0.1	3.20	1.5

The results show that the whole GSE, PPC and OPC fractins had high activity against all virulent *H. pylori* strains.

For GSE, the reduction of log CFU/mL was from 2.87 to 5.79, depending on the *H. pylori* strain, and MIC values were from 0.075 to 1.5 mg/mL.

The PPC-rich fraction had the highest antibacterial activity, showing a log reduction of CFU/mL from 2.19 to 4.89 and MIC values from 0.075 to 0.1 mg/mL.

In contrast, the activity of OPC-rich fraction had the lowest values of log reduction of CFU/mL and the highest MIC values.

References
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