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

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MAIN CONCLUSION

The whole GSE and the OPC-rich and PPC-rich fractions had high 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.

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. 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.

MATERIALS & METHODS

PPC-rich and OPC-rich fractions were obtained by a 10 kDa ultrafiltration membrane and preparative solid-phase extraction (SPE) on a XAD7HP/XAD16 adsorbent resins [3]. Chemical characterization of GSE, PPC and OPC-rich fractions was based on the determination of total phenolic content (TPh) by Folin-Ciocalteu assay, total procyanidin content (TPC), by acid butanol assay, total carbohydrate content (TCH) by GC-FID-MS and total catechins, total OPC and total PPC by NP-HPLC-PAD in a semi-quantitative way [1]. Antibacterial activity of GSE and its fractions against *H. pylori* strains was evaluated following the procedure described in [4].

RESULTS & DISCUSSION

The composition of GSE, OPC-rich and PPC-rich fractions is shown in Table 1.

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
Total phenolic (TPh)	25.1 ± 0.5	34.9 ± 0.5	49.5 ± 1.3
Total procyanidin (TPC)	8.5 ± 0.3	14.6 ± 0.5	12.9 ± 0.4
Total carbohydrate (TCH)	10.5 ± 0.2	3.6 ± 0.2	0.36 ± 0.02

TPh content is expressed as equivalents of gallic acid; TPC content is expressed as equivalents of cyanidin; TCH is a sum of all monomer and dimer saccharides, and polyols obtained by GC-FID.

The TPh content indicates that both PPC and OPC fractions contained almost 1.4 and 2-fold higher amounts of phenolic compounds than the GSE. On the other hand, the TPC showed that higher enrichment of procianydins was achieved in the PPC-rich fraction (1.7-fold) than the OPC-rich fraction (1.5-fold), but in both cases, it was lower than 2-folds. This difference could be explained by the high sensitivity of the Folin–Ciocalteu assay to other grape seed constituents, such as reducing sugars, that were present at higher amounts in the GSE (10.5 g/100 g).

Results from NP-HPLC show that major components of the GSE were PPC (84%), whereas catechins and OPC were at lower proportion (6 and 9%, respectively). Separation of the macromolecular fraction allowed enrichment of PPC up to 96% of the total flavan-3-ol content, converting this fraction of highly purified PPCs. Purification of the low molecular mass components by SPE allowed the recovery of a fraction enriched in catechins and OPCs up to 58% of its total flavan-3-ol content. Nevertheless, 42% of PPC remained present in this fraction.

The antibacterial activity of the GSE and its OPC and PPC fractions against 6 *H. pylori* strains is presented in Table 2.

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

MIC: minimal inhibitory concentration, log CFU/mL values in the same row marked with different letters indicate significant differences by ANOVA post hoc LSD Tukey test (p≤0.05).

The results show that the whole GSE and the corresponding fractions enriched in OPC and PPC had high activity against *H. pylori*. Reduction of log CFU/mL was from 2.87 to 5.79, depending of the *H. pylori* strain. MIC values of GSE 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 was between 1.24 and 3.20 log CFU/mL, depending of the growth of the different *H. pylori* strains, with MIC values higher than those of the PPC-rich fraction. Further studies should attempt to clarify which of the two factors (larger polymer size or higher procyanidin content) is most relevant in the antibacterial activity against *H. pylori*.

REFERENCES

[1] Brown, JC; Huang, GH; Haley-Zitlin, V; Jiang, XP, *Appl. Environ. Microbiol*., 2009, 75, 848
[2] Silvan, JM; Gutiérrez, A et al., *Foods*, 2020, 9, 1370
[3] Gutierrez, A; Almodóvar, P; Moreno, S, *Membranes*, 2020, 10, 147
[4] Silvan, JM; Mingo, E; Hidalgo, M et al., *Food Control*, 2013, 29, 25