Gunnera perpensa ellagitannins synergistically inhibit multiple SARS-CoV-2 variants (*including omicron)*

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BACKGROUND: SARS-CoV-2 antivirals are urgently needed to supplement vaccine efforts and target variants of concern (VOC), particularly in resource-limited regions and against highly-transmissible VOC such as Omicron. Pure natural compounds may block SARS-CoV-2 cellular entry by disrupting the binding of the viral spike receptor binding domain (RBD) with the host angiotensin-converting enzyme II (ACE2) receptor. As the medicinal plant *Gunnera perpensa* L. is in use by South African traditional healers to manage suspected COVID-19, we hypothesized that it contains compounds that block SARS-CoV-2 VOC entry and replication. Here we describe isolation of punicalin (PC) and punicalagin (PG) from *G. perpensa* and demonstrate their ability to inhibit RBD-ACE2 and SARS-CoV-2 across multiple VOC including Omicron.

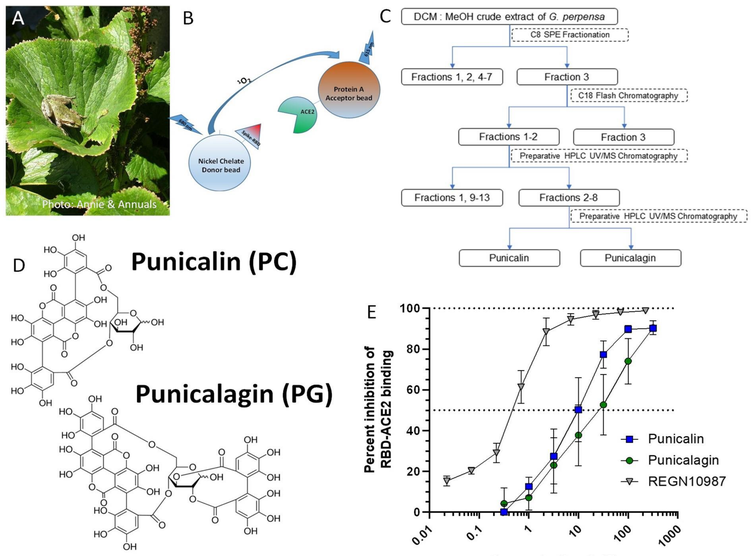
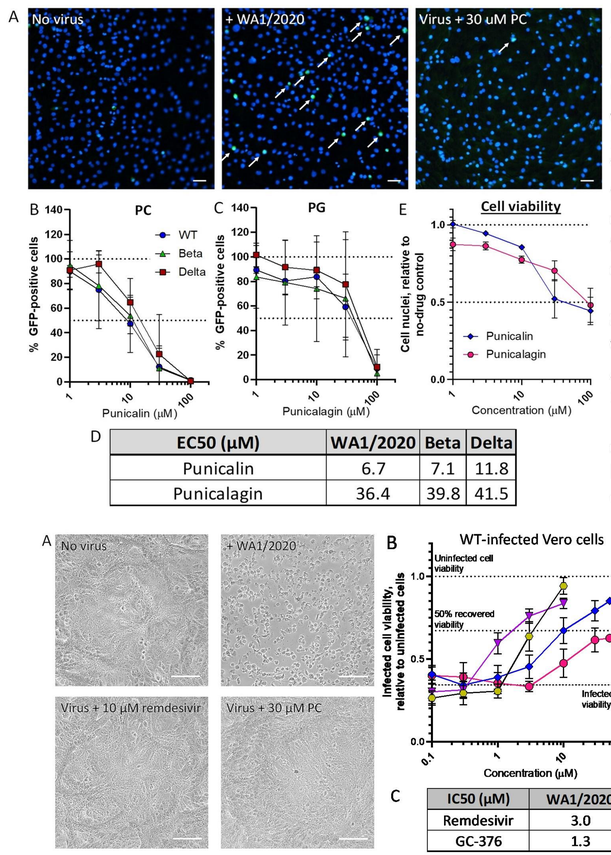


Figure 1. Isolation of PC and PG as RBD-ACE2 antagonists. A, *Gunnera perpensa*. **B,** AlphaScreen-based technology was used to measure RBD-ACE2 binding (PMID: 34543092). **C,** Summary of bioassay-guided fractionation steps to identify PC and PG (**D**). **E,** PC and PG both inhibit WT (USA-WA1/2020) RBD-ACE2 (IC_{50}s = 9 and 29 nM); IC_{50} of control antibody REGN10987 = 0.18 nM (25 ng/mL).



D EC50 (μM)	WA1/2020	Beta	Delta
Punicalin	6.7	7.1	11.8
Punicalagin	36.4	39.8	41.5

C IC50 (μM)	WA1/2020	IC50 (μM)	WA1/2020
Remdesivir	3.0	Punicalin	11.6
GC-376	1.3	Punicalagin	46.8

Figure 4. PC and PG inhibit live SARS-CoV-2. Vero-E6 cells were treated with PC, PG, or control antivirals remdesivir or GC-376 for 2 h followed by WT SARS-CoV-2 infection (150x TCID50). **A,** After 96 h, infected cells have widespread CPE but not when treated with inhibitors. **B,** When stained with resazurin, a cell viability dye, infected cells have 65.7% reduced viability relative to uninfected cells (top and bottom dotted lines). In contrast, control compounds, PC, and PG restore cell viability with dose-dependence, consistent with antiviral activity. **C** shows calculated EC50s with this assay. **D,** No major effects on uninfected cell viability are observed by compounds after 96 h culture.

Figure 3. PC and PG inhibit transduction of VOC spike pseudoviruses. ACE2-expressing BHK21/WI-2 cells were transduced with VSVΔG-S-GFP pseudoviruses (PMID: 20709108) with different VOC spikes ± PC or PG in 384-well format. After 24 h, cells were counted for total nuclei (Hoechst, for cell viability) and GFP-positive cells (pseudovirus transduction) by high-content imaging (PMID: 34543092). **A,** Cells transduced with WT spike pseudovirus ± PC. Arrows show some GFP. Both PC **(B)** and PG **(C)** inhibit pseudovirus regardless of VOC spike in live cells. **D** shows estimated EC50s. **E,** Pseudovirus block is independent of cell viability.

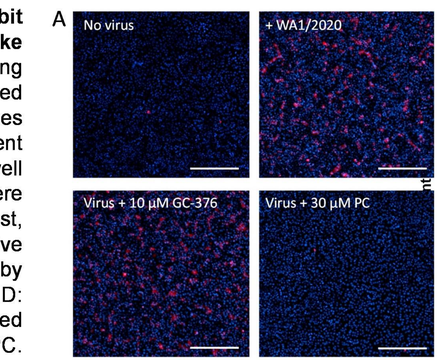
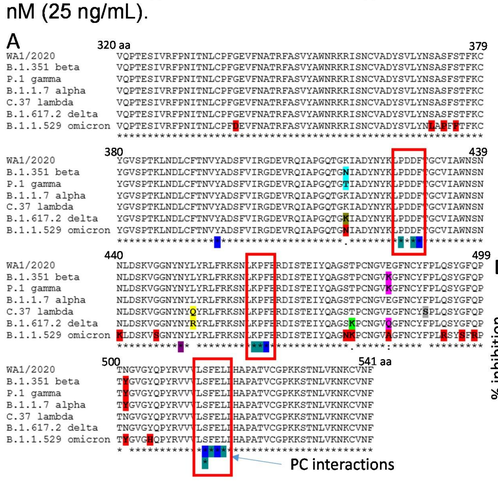
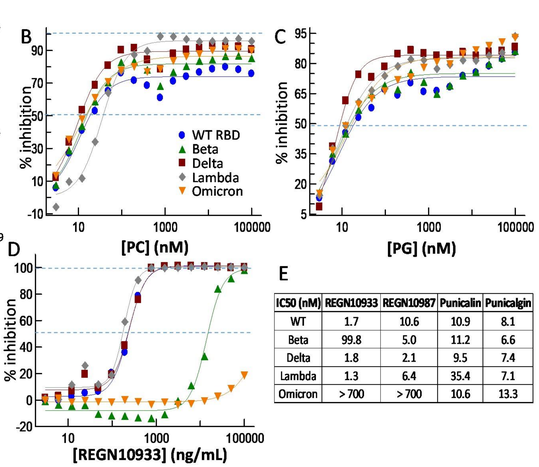


Figure 5. PC and PG inhibit VOC. Vero-E6 cells were treated with compounds and infected with WT, beta (B.1.1.7), or delta (B.1.617.2) virus (50X TCID50). After 48 h, cells were fixed, stained for viral nucleocapsid (red) and nuclei (blue), and counted for infected and total cells by high-content imaging. **A,** Widespread nucleocapsid-positive cells occur following infection, but not with 30 μM PC. **B,** PC and PG inhibit all viruses with dose-dependence.

Mix	EC50 (μM)
PC	14.2
10:1 PC:PG	5.0
3:1 PC:PG	8.4
1:1 PC:PG	19.9
1:3 PC:PG	12.1
1:10 PC:PG	16.3
PG	24.9

Figure 6. PC:PG combinations enhance antiviral activity. Vero-E6 cells were treated with PC, PG, or PC:PG ratios before WT SARS-CoV-2 infection using the assay in Figure 3B. Both 1:10 and 3:1 PC:PG mixtures enhance activity relative to PC or PG alone. *'s denote significant synergism ($p < 0.05$; paired Student's test) using the Bliss Independence model (PMID: 32788215).





E IC50 (nM)	REGN10933	REGN10987	Punicalin	Punicalgin
WT	1.7	10.6	10.9	8.1
Beta	99.8	5.0	11.2	6.6
Delta	1.8	2.1	9.5	7.4
Lambda	1.3	6.4	35.4	7.1
Omicron	> 700	> 700	10.6	13.3

Figure 2. PC and PG disrupt RBD-ACE2 across VOC by AlphaScreen. A, Alignment of RBDs indicates that VOC mutations are distinct from PC interaction sites (PMID: 33200379; 34246969; 34604010). Consistent with this, both PC **(B)** and PG **(C)** inhibit RBD from all assessed VOC with similar activities. In contrast, control antibody REGN10933 has variable activity across VOC as previously reported (PMID: 33684923; 34543092). **E** shows IC_{50}s.

CONCLUSIONS

- PC and PG are isolated from *Gunnera perpensa*, a medicinal plant traditionally used to treat suspected COVID in South Africa.
- PC and PG disrupt RBD-ACE2 binding, regardless of VOC sequences including SARS-CoV-2 Omicron RBD
- PC and PG also inhibit VOC pseudoviruses and multiple live viruses (WT, Beta and Delta; Lambda and Omicron in progress)
- Antiviral effects of PC and PG synergize in some combinations
- Result support *G. perpensa,* PC, and PG as antiviral leads against COVID, including due to emerging and highly-transmissible VOC, and to support ongoing vaccination and therapeutic efforts.

FUNDING SUPPORT

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