Supplementary Files

Supplementary Methods

Gel Permeation Chromatography

The purified sample (1 mg) was dissolved in 1 ml dimethylformamide (DMF) containing 50 mM lithium chloride (LiCl). Then, 200 µl of the sample was subjected to gel permeation chromatography (GPC). The molecular mass was estimated using a high-performance liquid chromatograph equipped with a ultraviolet (UV) detector (2489 UV/visible detector; Nihon-Waters, Tokyo, Japan) directly connected to a TSK gel α-3000 column (7.8 mm × 30 cm; TOSOH, Tokyo, Japan). DMF containing 50 mM LiCl was used as the solvent at a flow rate of 0.7 ml/min, and the column temperature was maintained at 40 °C. The peak top of the eluted sample was used for molecular mass determination, with a calibration curve obtained using standard polystyrene kits (PSt Quick E and F; TOSOH, Tokyo, Japan).

Antiviral Assay

The proanthocyanidin (PAC) solution or distilled water (the control) was sprayed onto the leaves of *Nicotiana benthamiana* plants in the third true leaf stage. The pTL-derived plasmids (pTLBN.G3), containing a full-length ToMV cDNA and a gene encoding green fluorescent protein (GFP) (Kubota et al. 2003), were used as a template for *in vitro* transcription using the AmpliCap-Max T7 High Yield Message Maker Kit (CELLSCRIPT, Madison, WI, USA). Three days after the treatment, the third true leaves of *N. benthamiana* were mechanically inoculated with 20 µl of 40-fold dilution of the transcription mixture. The antiviral activity was assessed based on the number of GFP foci formed on the treated *N. benthamiana* leaves compared with those on the control. The number of GFP foci formed on the inoculated leaves was calculated under blue light irradiation at 3 days post-inoculation. Lastly, the protective value was calculated as (1 − number of GFP spots formed on treated plants / number of GFP spots formed on untreated plants) × 100.

Cytopathic Effect (CPE) Assay for Influenza A Virus

During the CPE assay for influenza A virus, Madin–Darby canine kidney (MDCK) cells were grown in high-glucose DMEM with L-glutamine and phenol red (Fujifilm Wako Pure Chemical, Japan) supplemented with 10% HyClone fetal bovine serum (FBS) (GE Healthcare, Japan) and 0.05 mg/ml kanamycin sulfate (Fujifilm Wako) in 96-well plates for 24 h at 36 °C under 5% CO_2. First, the PACs were added to pure water to prepare 0.1 to 1 mg/ml solutions. Then, 900 μl of each test solution and 100 μl of influenza A virus (H1N1 A/PR/8/34) preparation were mixed for 10–30 min. Next, 10× dilutions were prepared by adding the cell culture medium (DMEM with 1% FBS and 0.05 mg/ml kanamycin sulfate) and 100 μl of the diluted mixture into each well of a 96-well culture plate seeded with virus-susceptible cells. The cells were further incubated for 4 to 7 days at 36°C under 5% CO_2, and CPE was observed under an inverted microscope. The 50% endpoint dilution ($TCID_{50}$/ml) was calculated by the Reed and Muench (1938) method.

Cytopathic Effect (CPE) Assay for Porcine Epidemic Diarrhea Virus (PEDV)

During the CPE assay for PEDV, Vero cells were grown in minimum essential medium (MEM) for 5 days at 37°C under 5% CO_2. Proanthocyanidins from *Alpinia zerumbet* (AzPACs) were added to pure water to obtain concentrations of 0.1–0.5 mg/ml. Then, 900 μl of each test solution and 100 μl of each PEDV preparation were mixed for 30 min. After mixing, 10× dilutions were prepared by adding MEM and 100 μl of the diluted mixture into each well of 96-well culture plate in which virus-susceptible cells were sheeted. The cells were further incubated for 5 days at 37°C under 5% CO_2, and the CPE was observed under an inverted microscope. The 50% endpoint dilution ($TCID_{50}$/ml) was calculated by the Reed and Muench (1938) method.

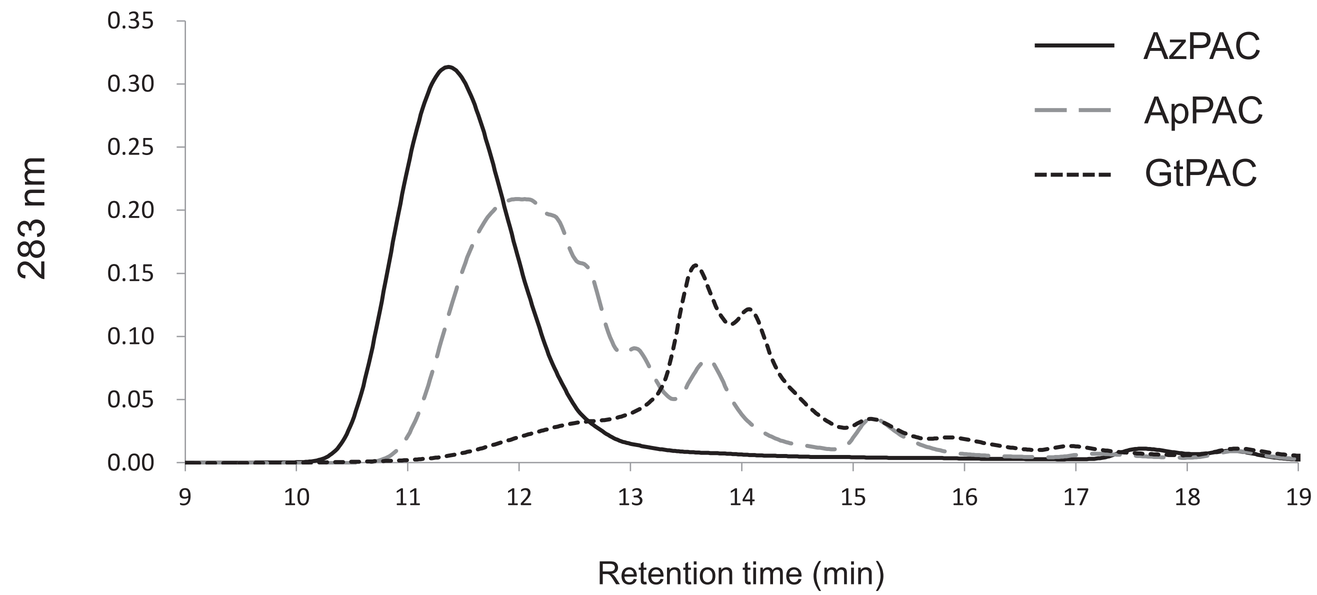
Statistical Analysis

52 The differences between the control and treated plants at each leaf position were analyzed using
53 one-way analysis of variance with Dunnett's multiple comparisons test. The differences with a *p*-
54 value of <0.01 were considered statistically significant.
55 The differences in protective values between treated plants were analyzed using one-way
56 analysis of variance with Tukey–Kramer multiple comparisons test. Differences with a *p*-value
57 of <0.05 were considered statistically significant.
58
59 **Supplementary Figure**
60 Supplementary Figure S1. The molecular mass distribution of proanthocyanidins from *Alpinia*
61 *zerumbet* (AzPAC), apple (ApPAC), and green tea (GtPAC). The purified sample (1 mg) was
62 dissolved in 1 ml dimethylformamide (DMF) containing 50 mM lithium chloride (LiCl), and 200
63 µl of the sample was subjected to gel permeation chromatography (GPC). The molecular mass
64 was estimated using a high-performance liquid chromatograph equipped with a UV detector
65 (2489 UV/visible detector; Nihon-Waters, Tokyo, Japan) and a TSK gel α-3000 column (7.8 mm
66 × 30 cm; TOSOH, Tokyo, Japan), which were connected directly. DMF containing 50 mM LiCl
67 was used as the solvent at a flow rate of 0.7 ml/min, and the column temperature was maintained
68 at 40°C. The peak top of the eluted sample was used for molecular mass determination, with a
69 calibration curve obtained using standard polystyrene kits (PSt Quick E and F; TOSOH, Tokyo,
70 Japan).
71
72 Supplementary Figure S2. Time-kill test of proanthocyanidin for influenza A virus. A solution
73 of 100 ppm of proanthocyanidins from *Alpinia zerumbet* (AzPAC) was mixed with or without
74 influenza A H1N1 virus (A/PR/8/34) for 10–20 min and added to MDCK cells. After incubation
75 for 4–7 days, a cytopathic effect was observed under an inverted microscope. The 50% endpoint
76 dilution ($TCID_{50}$/ml) was calculated using the Reed and Muench (1938) method.
77

78 **References**

79 Kubota K, Tsuda S, Tamai A, Meshi T (2003) Tomato mosaic virus replication protein
80 suppresses virus-targeted posttranscriptional gene silencing. *J Virol* 77: 11016-11026
81 Reed LJ, Muench H (1938) A simple method of estimating fifty percent endpoints. *Am J Hyg* 27:
82 493-497
83

Supplementary Figure S1



Supplementary Figure S2