帯広畜産大学
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Analysis of various virucidal substances against multiple pathogenic viruses

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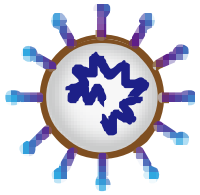
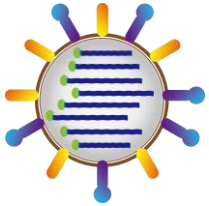
Pathogenic viruses in human



Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

-As of November 2022, more than 630 million infections and 6.6 million deaths have been reported.

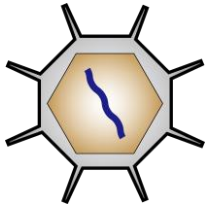
-There is a risk of emergence of new variant strains.



Influenza A virus (IAV) infection

-Many people are affected by global seasonal epidemics.

-There is a risk of emergence of novel pandemic strains.



Norovirus infection

-It is estimated that about one-fifth of acute enteritis worldwide is caused by this virus each year.

-Vaccines and therapeutic drugs have not yet been developed.

The social demand for development of novel virucidal agents which can safely and efficiently inactivate multiple pathogenic viruses is increasing.

Saxifraga plants

The use of naturally-derived components is currently attracting attention, as the basis for novel safe and eco-friendly virucidal agents.

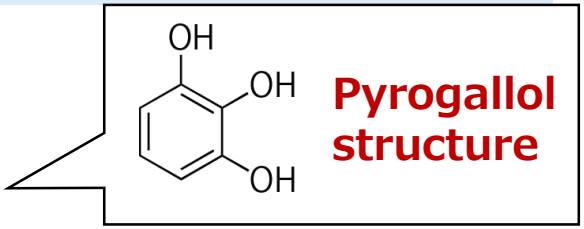
S. stolonifera

Saxifraga stolonifera

-is distributed in East Asia, including Japan.

-is edible and a medicinal plant, which has been used for treating inflammatory diseases.

Previous study of *Saxifraga* species



-A pyrogallol-enriched initial fraction obtained from the extract of Mongolian *S. spinulosa* showed potent virucidal activity against multiple pathogenic viruses. (Takeda, *Viruses.* 2020.)

The aim of the study

To identify the compounds responsible for the virucidal activity of *Saxifraga* species-derived fractions and elucidate its mechanism of action.

Methods: Preparation of *Saxifraga*-derived fractions

(Performed by Dr. Toshihiro Murata, Tohoku Medical and Pharmaceutical University)

Plants: five Japanese *Saxifraga* species

- ***S. stolontifera* (*Sst*)**
- ***S. fortunei* (*Sf*)**
- ***S. rebunshirensis* (*Sr*)**
- ***S. nipponica* (*Sn*)**
- ***S. cortusifolia* (*Sc*)**

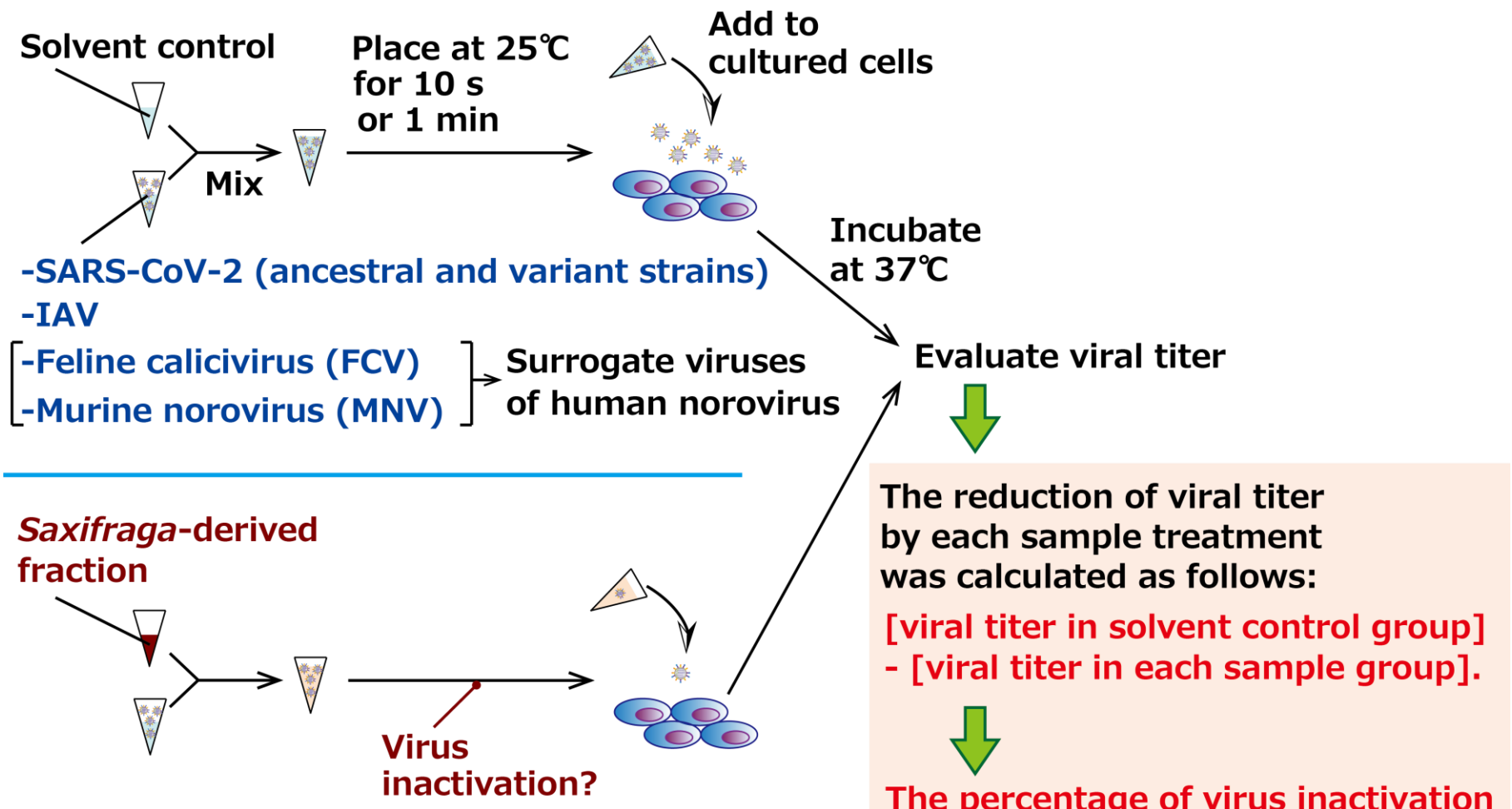
Crude extracts were extracted using 80% acetone.

The pyrogallol-enriched initial fractions were eluted with
-40% MeOH (fraction name: *Sst*-1A, *Sf*-1A, *Sr*-1A, *Sn*-1A, *Sc*-1A)
-60% MeOH (fraction name: *Sst*-1B, *Sf*-1B, *Sr*-1B, *Sn*-1B, *Sc*-1B)
from column chromatography using resin.

Sst-1A was further fractionated using reversed phase HPLC.

Twenty secondary fractions (*Sst*-2A－2T) were obtained.

Methods: Evaluation of virucidal activities of *Saxifraga*-derived fractions



Result-1: Virucidal activities of *Saxifraga* initial fractions

Values in each column:
The percentage of virus inactivation by each *Saxifraga*-fraction treatment

Target*→	SARS-CoV-2 (Ancestral)	IAV	FCV	MNV
Sample conc.→	25 μg/ml	25 μg/ml	25 μg/ml	100 μg/ml
Reaction time→	10 s	10 s	10 s	1 min
Sst-1A	≥99.99%	≥99.68%	97.66%	94.38%
Sst-1B	≥99.97%	≥98.68%	98.22%	96.84%
Sf-1A	≥99.97%	94.38%	97.66%	92.59%
Sf-1B	≥99.99%	≥98.68%	98.22%	97.66%
Sr-1A	≥99.98%	≥98.68%	99.99%	90.00%
Sr-1B	96.84%	87.41%	99.99%	68.38%
Sn-1A	98.68%	68.38%	96.02%	63.69%
Sn-1B	99.58%	N.S.	96.84%	63.69%
Sc-1A	≥99.97%	≥99.00%	96.84%	95.83%
Sc-1B	≥99.97%	98.22%	97.86%	N.S.

□ *$p<0.05$ ■ Not significant: N.S.

Almost all of initial fractions at 25 or 100 μg/ml showed virucidal activities against four different virus species in 10 s or 1 min.

Result-2: Virucidal activities of *Saxifraga* secondary fractions

Target*→	SARS-CoV-2 (Ancestral)	IAV	FCV	MNV
Sample conc.→	25 μg/ml	25 μg/ml	25 μg/ml	100 μg/ml
Reaction time→	10 s	10 s	10 s	1 min
Sst-2A－2K	N.S.	N.S.	N.S.	N.S.
Sst-2L	94.99%	N.S.	N.S.	N.S.
Sst-2M	96.84%	N.S.	98.74%	N.S.
Sst-2N	99.50%	99.00%	99.50%	96.84%
Sst-2O	99.21%	98.74%	99.50%	94.99%
Sst-2P	99.21%	92.06%	96.84%	N.S.
Sst-2Q	99.50%	≥99.87%	99.21%	99.00%
Sst-2R	≥99.93%	≥99.90%	99.68%	98.22%
Sst-2S	99.50%	≥99.68%	98.74%	98.74%
Sst-2T	99.00%	≥99.60%	98.74%	N.S.

□ *$p<0.05$ ■ Not significant: N.S.

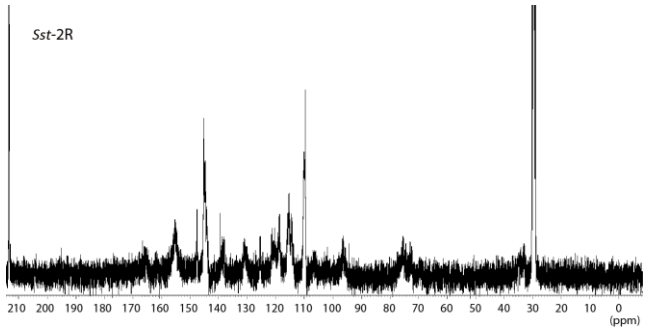
The virucidal activity of *Sst*-2R was the strongest.

↓

***Sst*-2R plays central role in the potent virucidal activity of *Saxifraga*.**

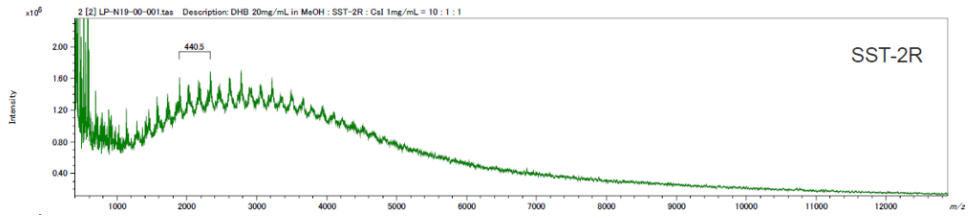
Result-3: Identification of condensed tannins

^{13}C NMR spectrum of *Sst*-2R

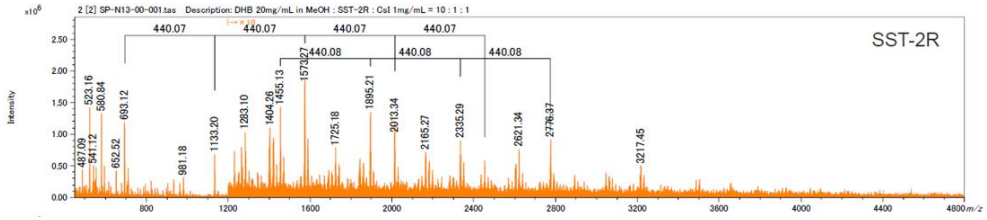


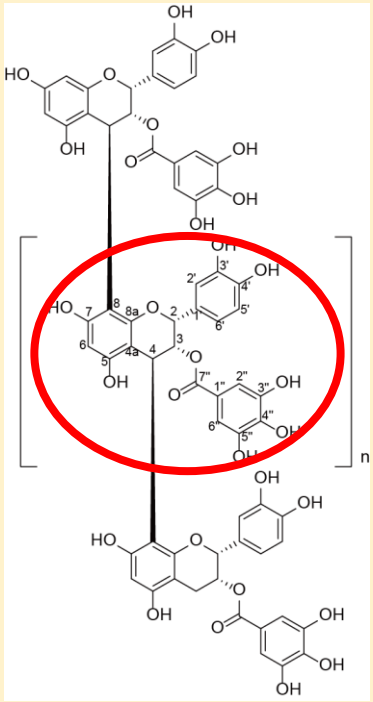
The spectrum of *Sst*-2R showed features of condensed tannins.
(Newman *et al.*, *Vmagn. Reson. Chem.* 1987.)

TOF-MS spectrum of *Sst*-2R (Linear mode)



TOF-MS spectrum of *Sst*-2R (Spiral mode)





Expected chemical structures of *Saxifraga* tannin.

- -The unit of the oligomer was epicatechin-3-*O*-gallate.
- -The 1−18 degree oligomers are present.
- -The 3−11 degree oligomers are main.

Result-4: Virucidal activities of *Saxifraga* initial fraction and condensed tannins against multiple SARS-CoV-2 variant strains

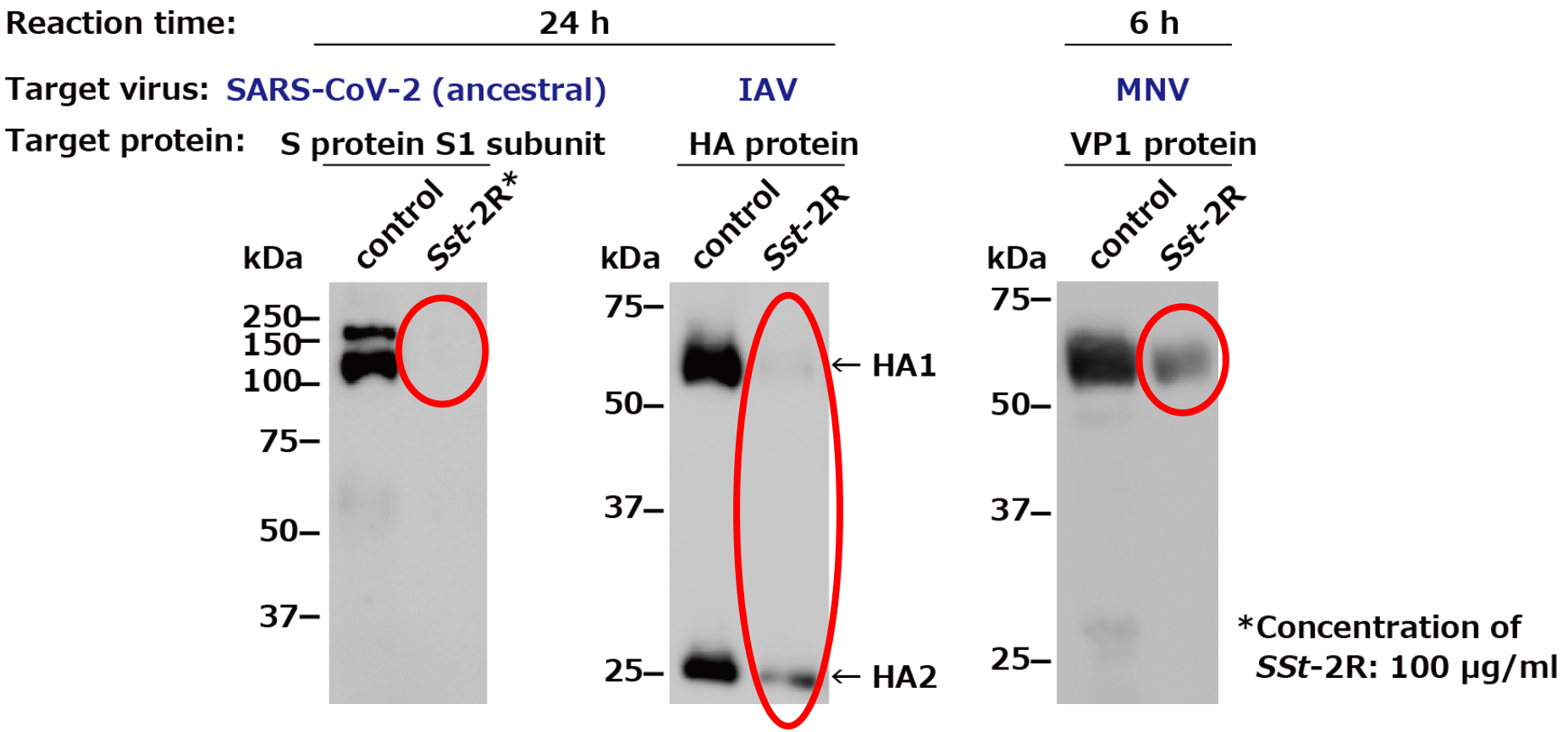
Target* (SARS-CoV-2)→	Alpha strain	Beta strain	Gamma strain	Delta strain	Omicron strain
Sample conc.→	25 μg/ml	25 μg/ml	25 μg/ml	25 μg/ml	25 μg/ml
Reaction time→	10 s	10 s	10 s	10 s	10 s
Sst-1A	≥99.90%	≥99.98%	≥99.99%	≥99.98%	≥99.84%
Sst-2R	≥99.99%	≥99.99%	≥99.99%	≥99.98%	≥99.98%

□ *$p<0.05$

The *Saxifraga* initial fraction and condensed tannins with 25 μg/ml induced ≥99.8% virus inactivation against all of tested variants in 10 s.

Result-5: Impact *of Saxifraga* tannin on viral structural proteins

The expression pattern of viral structural proteins on solvent control- or *Sst*-2R-treated viruses were analyzed using western blotting.



The disappearance or reduction of band intensity of multiple viral structural proteins was observed following *Sst*-2R treatment.

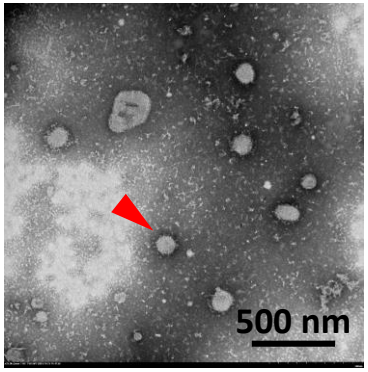
⇨ *Saxifraga* tannin induced structural abnormality of virus proteins.

Result-6: Morphology of *Saxifraga* tannin-treated virus particles

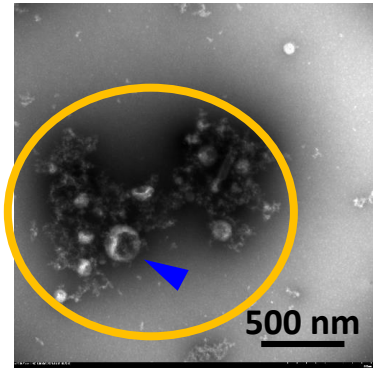
Solvent control- or *Sst*-2R-treated virus particles were directly observed using transmission electron microscope (TEM).

Target: Bovine coronavirus (surrogate of SARS-CoV-2)

control



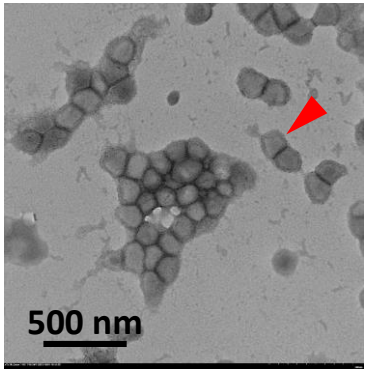
Sst-2R



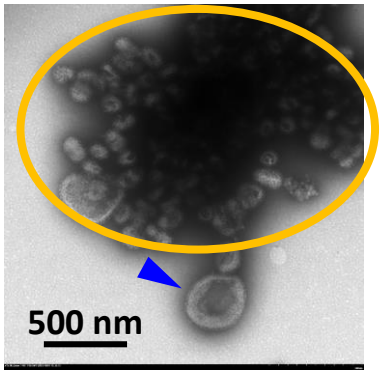
- : Intact particles (virus with normal size, clear spike protein, envelope, and/or capsid)
- : Abnormal particles (virus with bigger size, disruption of envelope or capsid)
- : Aggregation of virus particles

Target: IAV

control

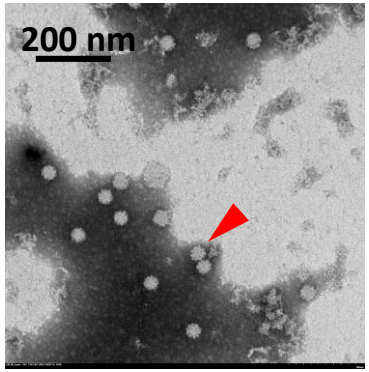


Sst-2R

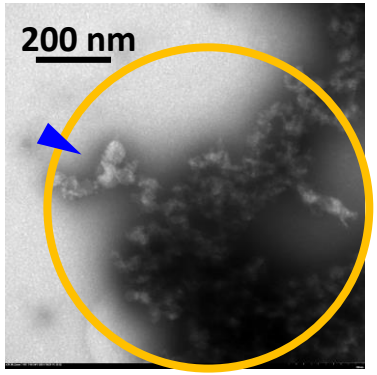


Target: MNV

control



Sst-2R



TEM analysis revealed that *Sst*-2R treatment induced morphological abnormality and aggregation of viral particles.

Conclusion

- Condensed tannins are the components which play a central role in virucidal activity of Japanese *Saxifraga* species.
- Condensed tannins induce structural abnormalities and aggregation of virus particles.
- *Saxifraga* species-derived fractions and condensed tannins show potent and rapid virucidal activity against multiple pathogenic viruses.

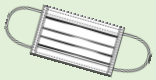
Possible applications of *Saxifraga* fractions/condensed tannins


Virucidal disinfectant


Antiviral hand cream


Troche


Mouth washer


Mask


Inhalant

***Saxifraga* species-derived fractions/condensed tannins can be used in practice as virucidal agents for multiple pathogenic viruses.**