Title

Anti-influenza virus activity of two extracts of the blackcurrant (ribes nigrum L.) from new zealand and poland

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ANTI-INFLUENZA VIRUS ACTIVITY OF TWO EXTRACTS OF
THE BLACKCURRANT (RIBES NIGRUM L.)
FROM NEW ZEALAND AND POLAND

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Abstract : We investigated the inhibitory effect of extracts of blackcurrant (Ribes nigrum L.) from
New Zealand and Poland on 4 strains of influenza virus (IFV) by the inhibition of virus adsorption ;
pandemic flu from 2009-2010 (IFV-AH1pdm), Hong Kong flu (IFV-AH3), oseltamivir phosphate-
resistant Russian flu (IFV-AH1tam') and influenza virus type B (IFV-B). The inhibitory effect of
the extracts of blackcurrant or blueberry on the infectivity of the virion were evaluated by the inhi-
bition of virus adsorption on the cell surface (adsorption-inhibitory assay). Three percent solutions
of the blackcurrant extracts from New Zealand and Poland were enough to disinfect more than half
of IFV-AH1pdm and IFV-B, and 10 % solutions from both regions disinfected all IFV strains com-
pletely. Our previous study showed that the antiviral effect of the blackcurrant differed according
to viral species. Here we showed that although the antiviral effect of Blackcurrant was slightly dif-
ferent within viral strains from one species, the extract of Blackcurrant could disinfect all of 4 IFV
strains we examined. The extracts of blackcurrant showed definite potential for use as a disinfec-
tant and antiseptic agent to prevent IFV infection.

Key words : antiviral agents, Blackcurrant, disinfectant, functional food

INTRODUCTION

Polyphenols in cranberries (Vaccinium macro-
carpon Aiton) and elderberries (Sambucus nigra L.)
are known to have the potential to prevent infec-
tious diseases. For instance, cranberries can pre-
vent gastrointestinal disorders associated withHelicobacter pylori3-5), reovirus6), rotavirus7) and influ-
enza virus8) infections, and elderberry inhibits the
replication of influenza virus9) and ease its symp-
toms6). Blackcurrant (Ribes nigrum L.) is also
known to inhibit the growth of herpes simplex virus
type19), the growth and release of influenza virus
type A and B10,11). Here we focused on the black-
currant’s preventive effect against influenza virus
infection by investigating the inhibitory effect
against viral adsorption. However, our previous
study showed that the antiviral effect (inhibition of
viral adsorption and replication) of the blackcurrant
differed according to viral species12). In order to
clarify whether this difference in effect is also
observed between strains from one species, the
inhibitory effects against pandemic flu from 2009–
2010 (IFV-AH1 pdm) and oseltamivir phosphate-
resistant Russian flu strain (IFV-AH1tam'), a newly
emergent strain, were investigated through a com-
parison with the IFV strains studied previously.

MATERIALS AND METHODS

Preparation of the fruit extracts

Blackcurrant and blueberry fruit products were
ground down and centrifuged at 1,600×g for 10 min
to remove insoluble contents and then stored at
−20°C until use. The pH of the extract was
adjusted from 2.8 to 7.2 using a sodium hydroxide
solution. On neutralization, some factors were

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observed as insoluble particles. These particles were precipitated as a pellet by centrifugation at 1,600×g for 10 min and the supernatant was used as the extract.

**Cell and viruses**

MDCK (Madin-Darby canine kidney) cells were cultivated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 4% fetal calf serum, antibiotics and antifungal agent (300 µg/ml of streptomycin, 300 U/ml of penicillin and 1 µg/ml of Amphotericin B). Four strains of influenza virus, pandemic flu from 2009/2010 (IFV-AH1pdm; A/Yamagata/165/2009pdm), Hong Kong flu (IFV-AH3; A/Yamagata/72/2009), oseltamivir phosphate-resistant Russian flu (IFV-AH1tamr; A/Yamagata/5/2009) and influenza virus type B (IFV-B; B/Yamagata/89/2009, Victoria lineage), were used in this study. All of the strains were isolated in the Yamagata Prefectural Institute of Public Health in Yamagata, Japan, in 2009. All of the viruses were made by growing in the MDCK cells. The cells were frozen and thawed twice to harvest as much of the viruses as possible. Viral titers were measured prior to the assays.

**Antiviral assays**

The inhibitory effect of the extracts of blackcurrant or blueberry on the infectivity of the virion were evaluated by the inhibition of virus adsorption on the cell surface (adsorption-inhibitory assay) as previously described. Confluent monolayers of MDCK cells in 12-well microplates were inoculated with 200, 2,000 or 20,000 pfu/well (Moi=4×10⁻⁴, 4×10⁻³ or 4×10⁻²) of the viruses and 0, 0.3, 1, 3 or 10% solutions of the blackcurrant or the blueberry at the same time. After adsorption for 5 min at 37°C, the inocula containing the virus and the solution were removed, and the cells were cultured in DMEM containing 1% Ultra Pure Agarose (Invitrogen, Carlsbad, CA) with 1 µg/ml of trypsin (Sigma-Aldrich, Co., St. Louis, MO) in a 5% CO₂ incubator. After 3 days of incubation, the cultures were fixed with 10% formaldehyde in phosphate-buffered saline, stained with 0.05% crystal violet, and the number of plaques was counted. The inhibitory effect was expressed as a percentage relative to that of the blackcurrant-negative control.

**RESULTS**

The 10% blackcurrant solution from both New Zealand (Figure 1A) and Poland (Figure 1B) completely inhibited adsorption of IFV-AH3 (closed circles), -AH1tam (open squares) and -B (closed squares). The 10% blueberry solution (Figure 1C) did not inhibit the adsorption of IFV-AH3 or IFV-AH1tam but did effectively inhibit IFV-AH1pdm (open circles) and IFV-B. The inhibition ratios were 0.0±0.0%, 39.0±5.9%, 97.8±2.2% and

![Fig. 1. Inhibitory effect of the blackcurrant extract on viral adsorption to cells.](image-url)
97.1±0.0%, respectively. Similar tendencies were obtained with the 3% blackcurrant solution, which effectively inhibited the adsorption of IFV-AH1pdm and IFV-B, but not IFV-AH3 or IFV-AH1tam.4

DISCUSSION

Our previous study showed that the blackcurrant has antiviral effects against several viral species, such as herpes simplex virus type19), respiratory syncytial virus, IFV-A and IFV-B21), but the antiviral effects of the blackcurrant differ between virus species, the 50% inhibitory concentrations (%) were 0.74±0.06, 0.18±0.06, 0.28±0.07 and 0.64±0.18, respectively. Therefore, in this study, we set out to determine whether the extracts of the blackcurrant have antiviral effects against several strains of IFV. Anthocyanins from blackcurrant are known to inhibit influenza virus adsorption to cells and also virus release from infected cells10). Here we focused on the preventive effect against influenza virus infection. Our particular focus was on whether the extract of blackcurrant can disinfect pandemic or oseltamivir phosphate-resistant IFV, both of which have been the cause of severe social unrest either through their spread across the globe due to the lacking specific immunities or difficulties in their treatment due to their oseltamivir phosphate-resistance. Although we could employ limited 4 strains of IFV as representatives, our results showed that a 10% blackcurrant solution had an antiseptic effect against multiple strains of IFV. Thus the blackcurrant extract has the potential to prevent infection with pandemic and drug-resistant IFV at least the strains that we employed.

We speculate two possible mechanisms for its antiviral effects. One possibility is that some component(s) in the blackcurrant affects the viral receptors on the cell surface, and the other possibility is that the component(s) affects IFV hemagglutinin (HA). Differences in the effects on IFV-AH1pdm and IFV-AH1tam, which possess similar HA1, indicate the former possibility. A previous report also showed that the pretreatment of cell cultures with cranberry juice could reduce reovirus infectivity43). However, the different effects of blackcurrant on the adsorption of the 4 IFV strains to the same MDCK cell line, support latter possibility as well. We already described that the blackcurrant has different antiviral effects against several viral species21). Another group also reported nonspecific antiviral effects towards unrelated viral species such as T2 and T4 bacteriophages and the simian rotavirus SA-1139. Taken together, these results suggest that the blackcurrant acts against both the viral and cellular surfaces. Although one of the promising candidates for the antiviral effects is anthocyanin, it is thought that there are many more candidates for the effective ingredient in the blackcurrant10).

Moreover, the benefits obtained from natural products might vary according to place of origin, and this may reduce the scientific reliability of the effects. We investigated blackcurrant extracts from different two regions, New Zealand and Poland. The blackcurrant extracts were from several farms in each region. Although we cannot exclude the possibilities that there are any other strains of blackcurrant with higher/lower antiviral effects in both regions, so far there were no significant differences in antiviral effects between the extracts from New Zealand and Poland, and the efficiency of both was better than the blueberry extract from North America used as the control.

In conclusion, the blackcurrant extract has the potential to prevent the adsorption of IFV, both of the strain from the pandemic in 2009-2010 to which very few people had immunity and of oseltamivir phosphate-resistant IFV, and could be used as a component in health care goods such as gargle products, candies and juices for the prevention of IFV infection.

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