



Inhibitory effect of grapefruit seed extract (GSE) on avian pathogens

Miyuki KOMURA¹⁾, Mayuko SUZUKI¹⁾, Natthanan SANGSRIRATANAKUL¹⁾, Mariko ITO¹⁾, Satoru TAKAHASHI¹⁾, Md. Shahin ALAM¹⁾, Mizuki ONO¹⁾, Chisato DAIO¹⁾, Dany SHOHAM²⁾ and Kazuaki TAKEHARA^{1)*}

¹⁾Laboratory of Animal Health, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan

²⁾Bar-Ilan University, Begin-Sadat Center for Strategic Studies, Ramat Gan 5290002, Israel

ABSTRACT. The inhibitory activities of grapefruit seed extract (GSE) on avian influenza virus (AIV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), *Salmonella* Infantis (SI) and *Escherichia coli* (EC) were evaluated. Original GSE contained 0.24% benzalkonium chloride (BZC), however, 0.0025% BZC solution could not inactivate bacteria. The activity of diluted GSE ($\times 100$, $\times 500$ and $\times 1,000$ with redistilled water) against selected viruses and bacteria was evaluated in this study. The GSE solutions were incubated with the pathogens over a period of time after which the remaining viruses were titrated and the bacterial colonies were counted. In the presence of organic material—5% fetal bovine serum (FBS), the test solutions were sprayed at 1 cm and 30 cm distances to test the efficacy of GSE in a spray form. Furthermore, the efficacy of GSE against bacteria on clothes was tested using non-woven cloth. GSE $\times 100$ reduced the viral titer of both AIV and NDV even in 5% FBS condition. IBDV showed high resistance to GSE. GSE $\times 1,000$ inactivated both SI and EC within 5 sec, even in the presence of 5% FBS. The disinfectant was able to maintain its efficacy in the spray form at 30 cm distance. GSE was also effective against SI and EC inoculated on fabric. GSE is a potential novel disinfectant against viruses and bacteria, effective even within a short contact time.

KEY WORDS: avian influenza virus, enhancement of biosecurity, grapefruit seed extract, spraying method

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Due to globalization, there are higher chances of the spread of zoonotic diseases by contagion. The enhancement of biosecurity is crucial, especially at ports such as airports, where people come and go, and at farms, where there is a higher chance of people having contact with animals. There are various disinfectants in the market, but the ones that are effective in various conditions and at the same time safe for animals are limited. For example, hypochlorous acid (HOCl) is safe for animals, and has a strong virucidal effect, but this effect is lost in its spray form at 30 cm distance [9]. Therefore, we considered the potential of grapefruit seed extract (GSE) as a novel disinfectant for animals and humans due to its desirable properties.

GSE is well known for its disinfecting property against bacteria. It has a high growth inhibition effect against gram negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*, as well as gram positive bacteria, such as *Staphylococcus* spp. and *Enterococcus* spp. [22]. Due to its high bactericidal effect, the application of GSE to fresh vegetables [38], food packaging [30], hypromellose gel [2], and many more commodities are being considered. GSE is considered a food additive because of its natural origin and safety [13]. However, there have been some issues with the safety and the bactericidal effect of GSE in the past. In the early 2000s, there were claims that GSE sold in the market contained benzalkonium chloride (BZC) [29], benzethonium chloride [28], and eighteen other preservatives [6]. GSE sold in Japan also contained high concentration of BZC and benzethonium chloride [25], and hence, awareness on ingredient labeling of all GSE products sold in Japan was promoted. These reports threaten the safety and efficacy of GSE.

The GSE used in the current study was checked for preservatives, and the amounts of benzethonium chloride and triclosan were at undetectable levels (tested by Mizuken Co., Ltd., Osaka, Japan). However, the solution (diluted with redistilled water (dW₂) $\times 100$) contained 0.0024% BZC. In order to confirm that this concentration of BZC would not contribute to the bactericidal effect of GSE in this experiment, the bactericidal activity of a diluted commercially available BZC solution was also evaluated.

In the present study, the effect of GSE was evaluated on avian influenza virus (AIV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), *Salmonella* Infantis (SI) and *Escherichia coli* (EC). Avian influenza is listed as one of the top three

*Correspondence to: Takehara, K.: takehara@cc.tuat.ac.jp

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