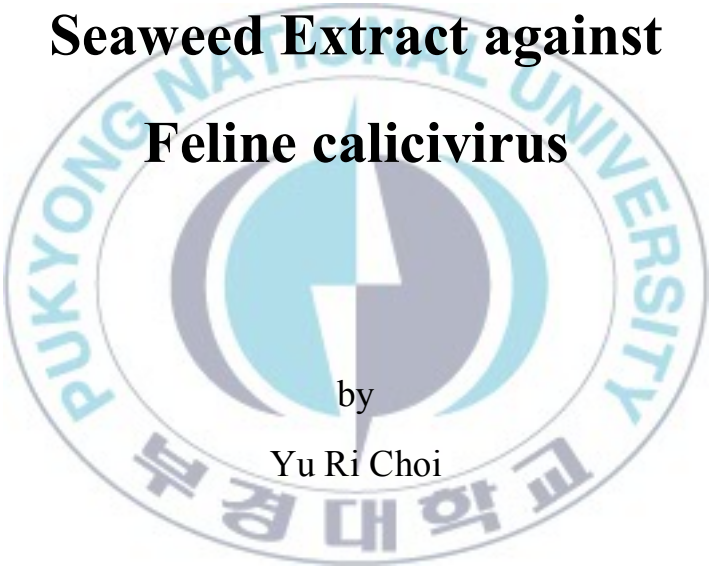
Thesis for the Degree of Master of Science

Effect of Antiviral Activity from Seaweed Extract against Feline calicivirus



by

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Effect of Antiviral Activity from Seaweed Extract against Feline calicivirus

(해조류 추출물의 Feline calicivirus 에 의한 항바이러스 활성)

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by

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Abstract

In decades, norovirus is getting attention as highly contagious viral infection disease and the most common cause of viral gastroenteritis in humans, and affects people of all ages. However, norovirus is difficult to investigate due to the deficiency of an in vitro cell culture. Thus relevant research is very limited.

The biological activities of natural extracts have been reported such as antiviral agent which is recognized safe but that has not been investigated. To investigate an antiviral substance against norovirus, several seaweeds extracts were evaluated for antiviral activity against feline calicivirus (FCV) as a norovirus surrogate.

Twelve different seaweeds (seven phaeophyta, two chlorophyta and three rhodophyta) were selected than examined its antiviral activity by using tissue culture infectious dose ($TCID_{50}$). As results, *Eisenia bicyclis* extract exhibited the highest antiviral activity against FCV inhibiting viral replication by 50% at a concentration of 80 μg/mL. The *E. byciclys* extract also showed the highest selective index (SI) value of 51.25, calculated from the ratio of the median cellular cytotoxicity concentration (CC_{50}) and effective concentration (EC_{50}). In addition, significant interruption of FCV infection was observed by treating Crandall-Reese feline kidney cells (CrFK), the host cell with *E. bicyclis* prior of virus infection in a dose-dependent manner. These results suggested that phaeophyta extract have potential as a considerable anti norovirus source.

Introduction

In recent decades, norovirus has been reported to cause mass food poisoning (Food Safety and Infection Service, 2009). According to the US Centers for Disease Control and Prevention, about 54 million people develop illness induced by norovirus annually in the United States and 96% of viral gastroenteritis occurred by secondary contamination (Cheek *et al.*, 2002; Koopmans and Duizer, 2004). In Korea, the first epidemic of food poisoning caused by norovirus outbreak in 1999 and norovirus was designated 'contagious disease' in 2006 (Jee *et al.*, 1999; Ministry of Health and Welfare, 2006). Although caution against norovirus diseases, number of norovirus related food poisoning outbreak have been increased in recent years (Korea Ministry of Food and Drug, 2012). KFDA also revealed that fishes and shellfishes are the major norovirus illness causative food (42%) and complex cooked food (20%), meat (20%) and water (7%) were followed.

Norovirus can infect human body through drinking water or food, and is then released into the environment via the vomit or feces. Then, it is transmitted by the ingestion of groundwater or contaminated food

with norovirus through the fecal-oral route (Fretz *et al.*, 2005). Spreading by the fecal-oral route, it is well-documented causes of food and waterborne diseases; their role in outbreaks associated with contaminated shellfish in particular the filter feeding oysters is infamous.

In 2012, norovirus was detected from oyster which was harvested from Nam-hae, the districted shellfish farm for exporting marine products to the United States, Europe and Japan. Eventually, United States rejected importing shellfishes from Korea. After many efforts to improve water quality and maintain its standard, exporting shellfished to USA was approved to resume in 2013. It is necessary manage water quality to inhibit norovirus contamination and it is needed to develop norovirus detecting technology to prevent norovirus infection (Ministry of Agriculture, Food and Rural Affairs, 2012).

Although boiling is an absolute safe way of eliminating pathogens and efficiently inactivates viruses, thermal processing may spoil the organoleptic qualities of shellfish. In order to satisfy consumers' preferences and food safety, non-thermal processing technology alternate thermal process minimizing organoleptic change and maintaining nutritional qualities. For instance, high-pressure processing

(HPP) is a representative non-thermal food processing technology that have preserved the appearance, flavor, texture, and nutritional value of ingredients (David *et al*., 2007). However, higher pressure treatment may cause demolish its appearance and texture of shellfish, also it can affect natural taste (Fangfei *et al*., 2011). Also, it is reported that norovirus shows resistance to several sanitizers that can induce various side effects, such as fever and itching, in humans (Liu *et al*., 2010; Park *et al*., 2010a; Centers for Disease Control and Prevention, 2011). Therefore, the development of effective and eco-friendly disinfectants against norovirus is necessary (Malik *et al*., 2006). Some disinfectant substances originating from natural sources are effective against bacteria and viruses. However, most natural substances are originated terrestrial plant for example ginseng, green tea, medicinal herbs, spices and terrestrial plants have strong flavor that does not blend in shellfishes (Park *et al*., 2005). Also, effective anti-norovirus agents from plants have been hardly reported. Therefore, it is assumed that seaweed could be suitable antiviral agents for shellfish which can maintain the characteristics of seafood and safe because they are collected from same habitat.

Recent norovirus studies utilized norovirus surrogates because norovirus is not cultivatable in laboratory conditions, and appropriate experimental protocols have not been established (Duizer *et al.*, 2004).

Thus, this study has focused on seaweeds, a potential antimicrobial additive and functional ingredient, revealed the antiviral activity of seaweed extracts against norovirus using FCV as a surrogate (Cho *et al.*, 1990; Kim *et al.*, 2009; Kim *et al.*, 2010; Smit *et al.*, 2004).

Materials and Methods

Sample preparation

Seaweed samples were obtained from a commercial market located in Busan, Korea in September 2012 (Table 1). Each sample was dried at 40°C for 2 days and then finely powdered. Each powder (100 g) was exhaustively extracted with aqueous 95% ethanol (v/v) at 70°C for 3 h. The ethanolic extract was filtered and concentrated by rotary evaporation at 40°C. The concentrated extract was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL.

Table 1. The seaweed species used for the tests of antiviral activities.

Species	Scientific name	Commercial name
Phaeophyta	*Hizikia fusiformis*	Seaweed fusiforme
	Laminaria japonica	Sea tangle
	Undaria pinnatifida	Sea mustard
	Sargassum fulvellum	Gulfweed
	Ecklonia cava	Kajime
	Ecklonia stolonifera	Gom pi
	Eisenia bicyclis	Sea oak
Chlorophyta	*Capsosiphon fulvescens*	Seaweed fulvescens
	Enteromorpha	Green lever
Rhodophyta	*Chondria crassicaulis*	-
	Gloiopeltis furcata	-
	Porphyra tenera	Laver

Cells culture

Crandall-Reese feline kidney cells (CrFK cells, ATCC CCL-94) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). CrFK cells suspended in Dulbecco's modified Eagle's medium (DMEM; GIBCO/BRL, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; GIBCO/BRL, NY, USA), and 1% penicillin-streptomycin (SIGMA, Dulbecco's, Germany) were seeded in 75 cm^2 culture flasks (SPL, Lifesciences, Korea) and incubated overnight at 37°C in humidified atmosphere 5% CO_2 incubator. Cells were subcultured every 2 to 3 days. The supernatant medium was discarded and the monolayer was washed with Dulbecco's phosphate buffered saline (PBS; SIGMA, Dulbecco's, Germany). A 1 mL volume of 0.05% trypsin-EDTA (GIBCO/BRL, Canada) was added to the monolayer. The flasks were incubated at 37°C about 2 min. About 10 mL of growth medium (DMEM/10% FBS) was added to the cells, centrifuged, and the cell suspension dispensed equally into culture flask. The flasks were incubated 5% CO_2 at 37°C for 2 days.

Virus culture

Feline calicivirus (FCV, ATCC VR-782) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The medium from CrFK cells in culture flask was inoculated the virus and were incubated 5% CO_2 at 37°C for 90 min. After virus adsorption, flask added about 10 mL of maintenance medium (DMEM/2% FBS) and incubated 5% CO_2 at 37°C for 16~24 h. FCV was propagated in CrFK cells and harvested by freeze and thawing twice, and centrifuged to remove cell debris. The virus suspension was stored at -80**°C** until use (Bidawid *et al.*, 2003).

Test for cytotoxicity of seaweed extract

The cytotoxicity of seaweed extracts was determined by quantifying the viability of CrFK cells using an MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as described by Kim *et al.* (2010). CrFK cells were seeded in 96-well plates at 1 x 10^5 cells/well and incubated for 24 h. Cell were treated with various concentration of seaweed extracts (0 – 1 mg) for 6 h. The medium was removed and 100 μL DMEM added to each well. 10 μL of MTT (Daeil Lab service, Seoul, Korea) was added and incubated for additional 3 h. The absorbance at 450 nm was measured with ELISA reader (Molecular Devices, Silicon Valley, CA, USA) and the inhibitory rates were calculated. The median cellular cytotoxicity concentration (CC_{50}) that resulted in the death of 50% of CrFK cells was determined by cell viability assay.

Plaque reduction assay for antiviral test

The antiviral activity of seaweed extracts against FCV was evaluated in terms of the plaque reduction assay. The cells were seeded in 6-well plates at 4 x 10^5 cells/well and incubated 5% CO_2 at 37°C for 2 days. After resulted in confluent monolayers, The FCV suspension (Log 5 $TCID_{50}$ per mL) was treated with identical volumes of serially diluted seaweed extracts at room temperature for 24 h. The mixtures were added to a monolayer of CrFK cells in a 6-well plate at least three wells. The plates were incubated 5% CO_2 at 37°C for 90 min and the mixtures was aspirated from each well which equal volumes of DMEM 2% FBS and 1.5% agarose was prepared. At the end of the virus incubation, each well added 2 mL of mixture medium. After the medium was incubated at 5% CO_2, 37°C. After incubating at 37°C for 2 to 3 days, the cells were then fixed by adding 2 mL per well of a 3.7% formaldehyde (JUNSEI, Japan). The solution was discarded and the cells were stained with 0.1% crystal violet solution (Kim *et al*., 2010), and monitored for CPEs.

The antiviral effective concentration was expressed as the effective concentration (EC_{50}) value, defined as the concentration of the sample

required to inhibit virus-induced cytopathogenic effects (CPEs) by 50% (Bidawid *et al.*, 2003). Untreated controls were suspended with maintenance medium instead of extract. The selectivity index (SI) was calculated as the ratio of CC_{50} to EC_{50} for each compound. The SI indicates the efficacy of antiviral activity. A higher SI means greater antiviral activity with low cellular toxicity, as well as higher commercialization potential (Oh *et al.*, 2013).

Tissue culture infectious dose$_{50}$ for antiviral test

The effect of seaweed extract on FCV infection was then evaluated in terms of the tissue culture infectious dose$_{50}$ ($TCID_{50}$) values (Kim *et al.,* 2010). The cells were seeded with 5 x 10^3 - 1 x 10^4 cells/well and added 100 μL each well, incubated 5% CO_2 at 37°C for 2 to 3 days. The FCV suspension was treated with identical volumes of serially diluted extracts at room temperature for 24 h. The mixtures were added to a monolayer of CrFK cells in a 96-well plate. Following 90 min incubation, mixtures removed by aspirate and 100 μL of maintenance medium were added to the each well. After incubating for 2 to 3 days, the cells were stained with crystal violet solution and monitored for CPEs. Untreated controls were suspended with maintenance medium instead of extract. The virus titer was estimated by the Reed-Muench method (Payment and Trudel, 1993).

Results and Discussion

Cytotoxicity of seaweed extract on CrFK cells

The cytotoxicity of the ethanolic seaweed extracts against CrFK cells was determined by MTT assay and reported as the CC_{50} value. The cells were treated various concentration of extracts. As a result, the *Gloiopeltis furcata* extract exhibited the lowest CC_{50} at 8.87 mg/mL (Table 2). The *Capsosiphon fulvescens*, *Enteromorpha* belong to chlorophyta showed a relatively low toxicity at 7.04 mg/mL, 7.14 mg/mL respectively. The *Sargassum fulvellum* exhibited the highest cytotoxicity at 3.24 mg/mL among twelve seaweed extracts. The CC_{50} value of ethanolic extract from *Undaria pinnatifida* was about a third of methanolic extract from *U. pinnatifida* (1.02 mg/mL) at 3.63 mg/mL (Kim *et al*., 2010).

Table 2. Cytotoxicity effects of seaweed ethanolic extracts on CrFK cells by Cell viability assay.

Species	Scientific name	CC_{50}[a] (mg/mL)
Phaeophyta	*Hizikia fusiformis*	6.36
	Laminaria japonica	4.37
	Undaria pinnatifida	3.63
	Sargassum fulvellum	3.24
	Ecklonia cava	6.20
	Ecklonia stolonifera	4.50
	Eisenia bicyclis	4.10
Chlorophyta	*Capsosiphon fulvescens*	7.04
	Enteromorpha	7.14
Rhodophyta	*Chondria crassicaulis*	3.49
	Gloiopeltis furcata	8.87
	Porphyra tenera	4.82

a 50% cytotoxic concentration (CC_{50}) is the concentration of the 50% cytotoxic effect.

Antiviral activity of seaweed extracts against FCV

Previous study suggested that some seaweeds exhibit antiviral activity against FCV (Kim *et al*., 2010). To screen effective antiviral seaweed source, twelve species samples were selected including seven species of phaeophyta, two species of chlrorophyta, three species of rhodophyta. The antiviral effective concentration was expressed as the effective concentration (EC_{50}) value. The lower EC_{50} value indicate superior to the antiviral activity at a low concentration extract.

As a result, phaeophyta exhibited relatively low EC_{50} by showing 0.08 mg/mL of *Eisenia bicyclis* extract and 0.21 mg/mL of *Ecklonia cava* and the *Hizikia fusiformis* extract inhibited FCV at 0.90 mg/mL). In the case of chlrorophyta, EC_{50} value of *C. fulvescens* extract and *G. furcata* extract was 1.20 mg/mL, 1.30 mg/mL respectively. The *G. furcata* extract showed antiviral activity at higher concentrations of 2.40 mg/mL. It is estimated that rhodophyta are less effective than phaeophyta or chlrorophyta (Table 3).

As shown in Table 3, *H. fusiformis* showed the lowest cytotoxicity (6.36 mg/mL) among the seven phaeophyta. *S. fulvellum, Ecklonia stolonifera*, *E. bicyclis*, *U. pinnatifida*, and *Laminaria japonica* also

showed relatively low cytotoxicity levels against CrFK cells, ranging from 3.2 to 4.5 mg/mL. The *S. fulvellum* extract exhibited the highest cytotoxicity at 3.24 mg/mL. Kim *et al.* (2009) reported that slightly higher cytotoxicity of the *E. cava*, *E. stolonifera*, *L. japonica*, and *U. pinnatifida* extracts at 1.82 mg/mL, 2.43 mg/mL, 0.49 mg/mL, 1.02 mg/mL respectively. This difference may be due to harvesting during a different season or the different origin of the raw materials (El-Masry *et al.*, 1995; Kulanthaiyesu and Sadaiyappa, 2012). Interestingly, the cytotoxicity of phaeophyta extracts against CrFK cells was lower than that of territorial plant extracts, including those of *Allium scorodorpasum*, *Capsicum annuum*, *Hovenia dulcis*, and *Citrus aurantium* (Kim *et al.*, 2009; Oh *et al.*, 2013), suggesting that the phaeophyta extracts exert a more positive effect on cell adherence and growth compared with extracts of medicinal herbs and spices.

A higher SI means greater antiviral activity with low cellular toxicity, as well as higher commercialization potential (Oh *et al.*, 2013). SI for *E. bicyclis* extract, calculated from the CC_{50} and EC_{50}, exhibited the highest SI, 51.25 respectively (Table 3). The *E. bicyclis* extract showed the lowest EC_{50} value (80 μg /mL), indicating that phaeophyta extract exhibits the highest anti-FCV activity, followed by *E. cava*, *E.*

stolonifera, *U. pinnatifida, L. japonica, S. fulvellum* and *H fusiformis.* In the case of chlrorophyta and rhodophyta was indicated lower antiviral activity than phaeopyta. This suggests that the *E. bicyclis* extract could be a suitable antiviral agent for use against FCV. The *E. cava* extract strongly inhibited plaque formation at low concentrations, 0.21 mg/mL respectively also showed relatively SI, 29.52 respectively, and the SI value of the *E. cava* extract was the lower of the *E. bicyclis* extract as it was highly cytotoxity on phaeophyta. However, these results are not consistent with previous finding that the *U. pinnatifida* extract exhibited the highest SI (20.4) (Kim *et al.*, 2010). This discrepancy may be a result of differences in the raw materials used in terms of harvest season and location, as described above (Kulanthaiyesu and Sadaiyappa, 2012). In addition, the SI of phaeophyta extracts were similar to those of territorial plant extracts with the exception of *Camelia sinensis*, ranging from 0.53 mg/mL to 6.29 mg/mL (Oh *et al.*, 2013). According to EC_{50} and SI values, antiviral activity of phaeophyta extract was superior than chlrorophyta extract and rhodophyta extract. As a consequence, it was estimated that phaeophyta extract is more effective antiviral source than chlrorophyta and rhodophyta.

This result that investigated the antiviral activities of seaweeds against FCV as a surrogate for norovirus, and found that *E. bicyclis* extracts of phaeopyta exhibited outstanding antiviral effects.

Fucoidan is a viscous polysaccharide component of the cell walls of phaeopyta such as *U. pinnatifida, L. japonica, H. fusiformis, E. cava, E. stolonifera* and *E. bicyclis* contained in the polysaccharide. (Park *et al*., 2010b). The molecular weight(average 100,000 to 2,000,000 Da) of fucoidan is very dependent on species and season of sampling. Also, contents of fucose and sulfate impact on physiological activity significantly (Berteau, O. and B. Mulloy, 2003). Fucoidan, polysaccharides are one of main constrituents of phaeopyta. The biological activities including anticoagulant (Koyanagi S *et al*., 2003), antiinflammatory (Cumashi A *et al.*, 2007), antitumor (Alekseyenko T *et al*., 2007), antivaral activities (Pack *et al*., 2010b). Therefore, fucoidan is expected the antiviral activity of phaeopyta by the physiologically active substance.

Table 3. Antiviral activity of ethanolic extracts from seaweed against feline calicivirus by plaque reduction assay.

Species	CC_{50} (mg/mL)	EC_{50}[a] (mg/mL)	SI[b]
Hizikia fusiformis	6.36	0.90	7.07
Laminaria japonica	4.37	0.60	7.28
Undaria pinnatifida	3.63	0.43	8.44
Sargassum fulvellum	3.24	0.72	4.50
Ecklonia cava	6.20	0.21	29.52
Ecklonia stolonifera	4.50	0.34	13.24
Eisenia bicyclis	4.10	0.08	51.25
Capsosiphon fulvescens	7.04	1.20	5.87
Enteromorpha	7.14	1.30	5.49
Chondria crassicaulis	3.49	1.30	2.68
Gloiopeltis furcata	8.87	2.40	3.70
Porphyra tenera	4.82	1.50	3.21

a 50% effective concentration (EC_{50}) is the concentration of the sample required to reduce plaque formation of virus by 50%.

b Selectivity index (SI) = CC_{50}/EC_{50}.

$TCID_{50}$ of seaweed extracts against FCV

The antiviral activities of phaeophyta extracts against FCV were measured by 50% tissue culture infectious dose, and expressed as $TCID_{50}$ values. To evaluate the protective effect against FCV infection on CrFK cells seaweed extracts were treated with various concentrations of ethanolic extracts prior to virus infection. According to $TCID_{50}$ values, antiviral activity of phaeophyta extract was superior than chlrorophyta extract and rhodophyta extract (Table 4). FCV was not completely inactivated the chlrorophyta and rhodophyta extracts. In the case of, *E. cava, E. stolonifera and E. bicyclis* extracts exhibited the strongest antiviral activity against FCV and seemed to inhibit the infectivity of FCV. Viral infection of CrFK cells was not observed after 24 h in the presence of 500 μg/mL and 1000 μg/mL of *E. cava, E. stolonifera and E. bicyclis* extracts. But other extracts did not completely inactivate FCV under these experimental conditions. An antiviral effect is assumed when a 4-log decrease occurs in the viral $TCID_{50}$/mL value (Bellamy, 1995). Extracts of *E. cava, E. stolonifera and E. bicyclis* caused about 5-log decrease value at 500 μg/mL indicating that these extracts are effective antiviral agents that can be

used to inhibit infection of FCV. A comparison of the SI values of *E. cava* (29.52), *E. stolonifera* (13.24) *and E. bicyclis* (51.25) extracts propose that the *E. bicyclis* extract is more useful than that of *E. cava* and *E. stolonifera* for controlling infection of CrFK cells by FCV (Table 3). The antiviral activity effect of another extracts were completely disregarded.

As a consequnce, it was estimated that phaeophyta extract is more effective antiviral source than chlrorophyta and rhodophyta. We suggest that seaweed extracts can be used instead of terrestrial plant for the effective control of norovirus.

Table 4. Time dependent antiviral activity of various ethanolic extract from seaweed against feline calicivirus for 24 h.

Spices	log ($TCID_{50}$/mL)[a]				
	0	10	100	500	1000
Hizikia fusiformis	4.9	4.6	4.4	4.4	4.4
Laminaria japonica	4.9	4.9	4.8	4.8	4.6
Undaria pinnatifida	4.9	4.9	4.9	4.8	4.9
Sargassum fulvellum	4.9	4.9	4.8	4.9	4.9
Ecklonia cava	4.9	4.8	4.6	Neg[b]	Neg
Ecklonia stolonifera	4.9	4.8	4.6	Neg	Neg
Eisenia bicyclis	4.9	4.6	4.6	Neg	Neg
Capsosiphon fulvescens	4.9	4.9	4.9	4.9	4.6
Enteromorpha	4.9	4.9	4.9	4.9	4.8
Chondria crassicaulis	4.9	4.9	4.9	4.9	4.9
Gloiopeltis furcata	4.9	4.9	4.9	4.9	4.9
Porphyra tenera	4.9	4.9	4.9	4.9	4.8

[a] $TCID_{50}$, 50% tissue culture infectious dose.

[b] Neg, feline calicivirus are inactivated.

Protective effect of seaweed extracts on FCV infection

Accordance to estimated EC_{50} value *E. cava, E. bicyclis* and *E. stolonifera* extracts on phaeophyta it is more effective antiviral source than chlrorophyta and rhodophyta. Accordingly, inactivated in the E. cava, *E. bicyclis* and *E. stolonifera* extracts to lower concentrations with repect to detail tested again. As shown in Figure 1, FCV infectivity was completely interrupted by treatment with 200 μg/mL *E. bicyclis* and *E. stolonifera* extracts. The *E. cava* extract also exhibited strong protective effect at 500 μg/mL. However, no significant protective effect against FCV infection was observed from treatment with *L. japonica*, *S. fulvellum*, and *U. pinnatifida*. Therefore, it is concluded that *E. bicyclis* also have protective effect norovirus infection.

Several reports of protection against viral infection have been published. Cho *et al.* (2013) reported a protective effect of red ginseng extract against vaginal herpes simplex virus infection, which was mediated by promotion of host defense systems. In addition, Carlucci *et al.* (2004) reported a protective effect of carrageenans against genital herpes simplex virus infection, which was mediated by interruption of viral replication. Although the precise mechanism of the protective

effect against FCV infection has not yet been defined, this results suggested that *E. bicyclis* extract inhibits viral penetration or replication (Carlucci *et al.*, 2004; Cho *et al.*, 2013). To address this issue, further studies are necessary to elucidate the mechanism underlying the protective effect of *E. bicyclis* against FCV infection.

To my knowledge, this is the first report of the protective effect of phaeophyta extract against FCV as a norovirus surrogate. It is believed theses result will contribute to the development of anti norovirus research and application to be food contact sanitizer effective against norovirus.

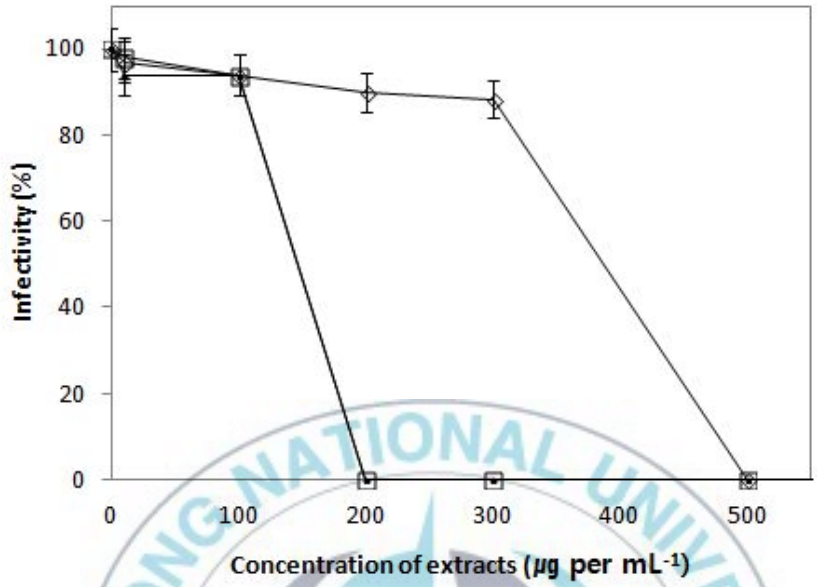

Fig. 1. Protective effect of three phaeophyta extracts on feline calicivirus (FCV) infection. Crandall-Reese Feline Kidney (CRFK) cells were treated with phaeophyta extracts for 24 h prior to FCV infection. Antiviral activity was determined by $TCID_{50}$ assay and the infectivity ratio was compared to control. ○, *Eisenia bicyclis*; □, *Ecklonia stolonifera*; ◆, *Ecklonia cava*

Conclusion

Norovirus is leading cause of gastroenteritis worldwide and are recognized as the most cause of food borne illness. Korea Ministry of Food and Drug also revealed that fishes and shellfishes are the major norovirus illness causative food. A recently, norovirus was detected from oyster which was harvested from Nam-hae. It is necessary manage water quality to inhibit norovirus contamination, and it is need to develop norovirus detecting technology to prevent norovirus infection. To find ways to effectively control the norovirus, a feline calicivirus (FCV) was used as a cultivatable norovirus surrogate and indicating antiviral activity of seaweed were investigated. Twelve different seaweeds (seven phaeophyta, two chlorophyta and three rhodophyta) were selected than examined its antiviral activity.

At the results, the *Eisenia bicyclis* extract showed the lowest EC_{50} value of 80 μg /mL, indicating that this extract exhibits the highest anti-FCV activity. The antiviral activity of seaweed extracts was then evaluated to determine SI value. The SI value of *E. bicyclis* extract calculated to the highest SI of 51.25. As a consequence, it was estimated that phaeophyta extract is more effective antiviral source than

chlrorophyta and rhodophyta. Fucoidan, biological activities including antivaral activities , antitumor, anticoagulant, antiinflammatory, is a viscous polysaccharide component of the cell walls of phaeopyta such as *U. pinnatifida, L. japonica, H. fusiformis, E. cava, E. stolonifera* and *E. bicyclis* contained in the polysaccharide. (Park *et al*., 2010b). Therefore, fucoidan is expected the antiviral activity of phaeopyta by the physiologically active substance.

Overall, this is the first report of the protective effect of phaeophyta extract against FCV as a norovirus surrogate. It is believed theses result will contribute to the development of anti norovirus research and application to be food contact sanitizer effective against norovirus.

국문 초록

노로바이러스는 지난 수십년동안 단체식중독 사고의 원인으로 보도되면서 새로운 병변으로 주목받고 있으며 국내에서 발생한 식품유래 질병의 가장 큰 원인으로 부각되고 있다.

전염성이 매우 높은 바이러스성 감염 질환으로 사람의 장에서만 증식되는 특성으로 현재까지 세포배양이 불가능하여 관련 연구가 미흡하고 또한 최근 안정성이 확보된 천연물 유래의 활성 물질에 대한 연구가 많이 보고되고 있으나 항바이러스 활성을 가지는 천연 물질에 대한 연구는 미약하다.

따라서 본 연구는 노로바이러스 저감화를 위해 해조류를 대상으로 Feline calicivirus (FCV)의 항바이러스 활성 효과를 확인하였다. 갈조류 7 종, 녹조류 2 종, 홍조류 3 종으로 총 12 종의 해조류를 선별하여 항바이러스 활성을 조사한 결과, 갈조류가 녹조류와 홍조류 보다 높은 활성을 보였으며, 그 중에서 대황 추출물의 EC_{50} 이 80 μg/mL, SI 값이 51.25 로 항바이러스 활성이 가장 우수하였다. 또한 대황은 200 μg/mL 농도에서 FCV 를 불활성화 시켰다. 이러한 결과는 갈조류가 잠재적으로

항노로바이러스 물질로 사용될 수 있으며 본 연구 결과는 노로바이러스를 저감화 할 수 있는 효과적인 식품 소독제의 발전에 기여할 것이다.

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