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Study on the medicinal plants against grouper iridovirus infection: A short communication

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Abstract. As one serious viral pathogen in mariculture, grouper Iridovirus (GIV) could cause high mortality to cultured groupers, then effective treatments against GIV infection are needed. The effects of *Thlaspi arvense* Linn. extracts against GIV infection was assessed by different methods in this study. The safe concentration of the water extract of *Thlaspi arvense* Linn. was initially determined as ≤ 1.25 mg/mL, which showed no obvious cytotoxic effects on cell viability. Using the safe concentration, aptamer(Q2)-based fluorescent detection assay and qRT-PCR results indicated that, *Thlaspi arvense* Linn. water extracts had excellent inhibitory effects against GIV infection *in vitro* and *in vivo*. In addition, the mechanism of *Thlaspi arvense* Linn. on the invasion process of the grouper iridovirus was examined, which showed that *Thlaspi arvense* Linn. water extracts had significant inhibitory effects at the stage of virus adsorption on the host cell membrane. The inhibitory rate was 87.48%. Overall, *Thlaspi arvense* Linn. water extracts have great antiviral effects, making it an interesting candidate for developing effective medicines for fighting against GIV infection in farmed groupers.

1. Introduction

Groupers are the commercial marine fish from the family *Serranidae* [1]. This high-quality edible fish contains low fat and high protein. Grouper are highly sought after for their meat quality and their delicious, tender flavor. Due to their high cost and economic value, grouper have become an important product in marine aquaculture in recent years especially in Asia [2-4]. According to the literature, most species of grouper in China are distributed in the South China Sea [1, 5]. As a result of significant



breakthroughs in reproductive growth regulations and artificial breeding technologies, the grouper industry in China has gradually entered a stage of large-scale aquaculture. However, various diseases challenging grouper populations in southern China have become increasingly serious with the expansion of caged aquaculture [5].

Grouper iridovirus is a serious infectious fish virus that has recently been discovered. It is also the main pathogen causing serious economic losses in grouper aquaculture. As a highly pathogenic infectious virus, grouper iridovirus can cause a large number of fish deaths in a short time [6, 7]. Hence, it is of great economic importance to discover a treatment that can inhibit the replication of the virulent grouper iridovirus that is currently challenging the grouper aquaculture industry [8]. Hence, the objective of the present study was to assess the inhibitory effects of *Thlaspi arvense* Linn. water extracts against grouper iridovirus infection.

2. Material and Methods

2.1. Virus and aptamer

This study used a strain of iridovirus that was isolated from cultured hybrid groupers (*E. fuscoguttatus*♀ × *E. lanceolatus*♂) from Guangxi (GIV-Gx) [6]. Aptamer Q2 for GIV-infected fish cells have been identified in the previous study [7]. Q2 was labeled by 6-carboxy-fluorescein (FAM), and served as molecular probe to detect GIV-Gx infection.

2.2. *Thlaspi arvense* Linn. water extracts preparations

For *Thlaspi arvense* Linn. water extracts preparations, dried *Thlaspi arvense* Linn. were smashed to be powder and macerated in water at 4 °C for 12 h. After boiled for 4 h, the mixtures were centrifuged at 8,000 g at 4°C for 40 min, the supernatant was collected and filtered through sterile nylon net filter (0.15 mm pore size).

2.3. Gene expression validation by qRT-PCR

The total RNA was reversely transcribed into cDNA by ReverTra Ace® qRT-PCR kit, then GIV infection were detected targeting the major capsid protein (MCP) by qRT-PCR. β -actin gene was used as an internal control. The average value of three independent experiments was calculated. Intergroup difference was compared by one way analysis of variance by SPSS statistical software (IBM, Armonk, NY, USA). The results of comparisons with $P < 0.05$ were considered to represent statistically significant differences.

2.4. Antiviral activity of *Thlaspi arvense* Linn. water extracts against GIV-Gx infection

GS cells were seeded in 24-well plate at 28 °C for 24 h. Then *Thlaspi arvense* Linn. water extracts (1.25 mg/mL) and GIV-Gx (MOI=0.5) were added into GS cells at 28°C for 48h. GIV-Gx alone (MOI=0.5) added into GS cells at 28°C for 48h served as the control group. At 48 hpi, cells and culture supernatants in each well were collected for total RNA extraction and analyzed by qRT-PCR. Aptamer(Q2)-based fluorescent detection assay by flow cytometer was also applied to analyze the antiviral activity of *Thlaspi arvense* Linn. water extracts against GIV infection.

3. Results and Discussions

China has a rich history in the use of Chinese herbal medicine to prevent and treat viral diseases. Such treatments are natural, efficient, inexpensive, less toxic, impart minimal side effects, and do not typically show drug resistance. In herbal remedies, the medicinal material itself contains alkaloids, glycosides, carbohydrates, organic acids, volatile oils, flavonoids, tannins, resins, oils, pigments, glycopeptides, amino acids and a variety of trace elements. These components act independently and synergistically. In addition,

these natural compounds supplement animal nutrition and promote growth, while also having anti-bacterial and immune effects. In recent years, herbal remedies have been more frequently used in aquaculture [9, 10].

Thlaspi arvense Linn. is a traditional Chinese medicine that was first published in the *Shennong Herbal Classic*. *Thlaspi arvense* Linn. is classified in the genus, *Thlaspi* L. and functions by clearing heat and detoxification, removing blood stasis and pus, and possesses anti-viral properties. Based on previous studies, *Thlaspi arvense* Linn. contains a variety of chemical components with a wide range of pharmacological effects and clinical applications [11]. In the present study, the aqueous extract of *Thlaspi arvense* Linn. was selected as the anti-iridovirus remedy for GIV-Gx infection in grouper.

Cell lines have been traditionally used in studying pathogenesis, immunology, and biotechnology development [12-15]. Toward this end, a grouper spleen cell line (GS), was used for these studies. A safe concentration of the water extract of *Thlaspi arvense* Linn. was initially determined [1]. The results showed that after 24 and 48 hours of incubation with >5 mg/ml water extract of *Thlaspi arvense* Linn., GS cells showed obvious pathological changes. However, an extract of 1.25 mg/ml or less had no obvious cytotoxic effects on cell viability. Using the safe concentration, the inhibitory activities of *Thlaspi arvense* Linn. aqueous extracts against GIV-Gx infection were analyzed by qRT-PCR and aptamer (Q2)-based fluorescent detection [16]. The results of qRT-PCR showed that the water extract of *Thlaspi arvense* Linn. had anti-iridovirus effects at 1.25 mg/ml. Aptamer(Q2)-based fluorescent detection was also applied to identify grouper iridovirus infection and the antiviral effects of *Thlaspi arvense* Linn., whose detective results were consistent with the qRT-PCR data. As aptamer(Q2)-based fluorescent detection is easy operation and could identify grouper iridovirus infection with high specificity, it had extensive applications in antiviral medicine development [17-22].

In addition, the mechanism of *Thlaspi arvense* Linn. on the invasion process of the grouper iridovirus was examined. It is well known that viral infection is a process in which the virus adsorbs to the cell membrane, passes through the host cell membrane, replicates in the cell, and finally releases from the host cell [23]. Therefore, understanding the influence of antiviral drugs on viral infection is of great significance for the development of antiviral drugs against grouper iridovirus. The results showed that the water extract of *Thlaspi arvense* Linn. had a significant inhibitory effect on the host cell membrane at the stage of virus adsorption. The inhibitory rate was 87.48%.

4. Conclusion

In conclusion, this is the first time the antiviral effects of *Thlaspi arvense* Linn. water extracts has been analyzed. The results showed that *Thlaspi arvense* Linn. water extracts had effective antiviral effects against grouper iridovirus, which is of great significance for the aquaculture industry to develop effective drugs to treat grouper iridovirus infections.

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