IDENTIFICATION OF *Artemisia annua* L. AS A POTENTIAL ANTI-VHSV AGENT THROUGH SCREENING OF NATURAL EXTRACTS

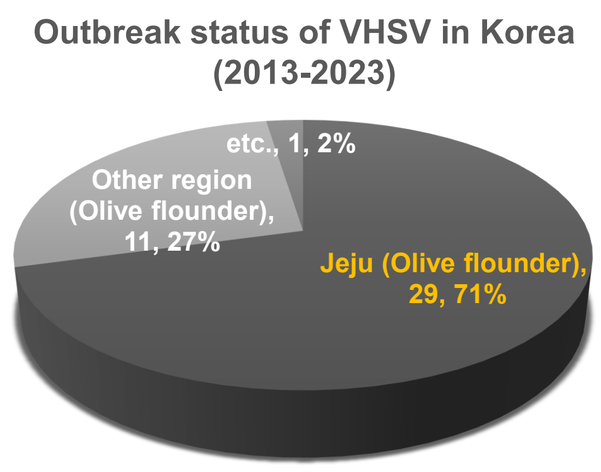
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Introduction

Viral hemorrhagic septicemia virus (VHSV) is a WOAH-listed aquatic pathogen that infects over 80 fish species, including economically important species such as olive flounder (Kurapati et al., 2026). It causes severe hemorrhagic disease and has led to significant losses in Korean aquaculture, particularly since its first report in 2001, with Jeju Island heavily affected. Despite ongoing vaccine research, practical application remains limited, and the absence of effective antivirals has led to concerns over antibiotic misuse (Priya & Kappalli, 2022).

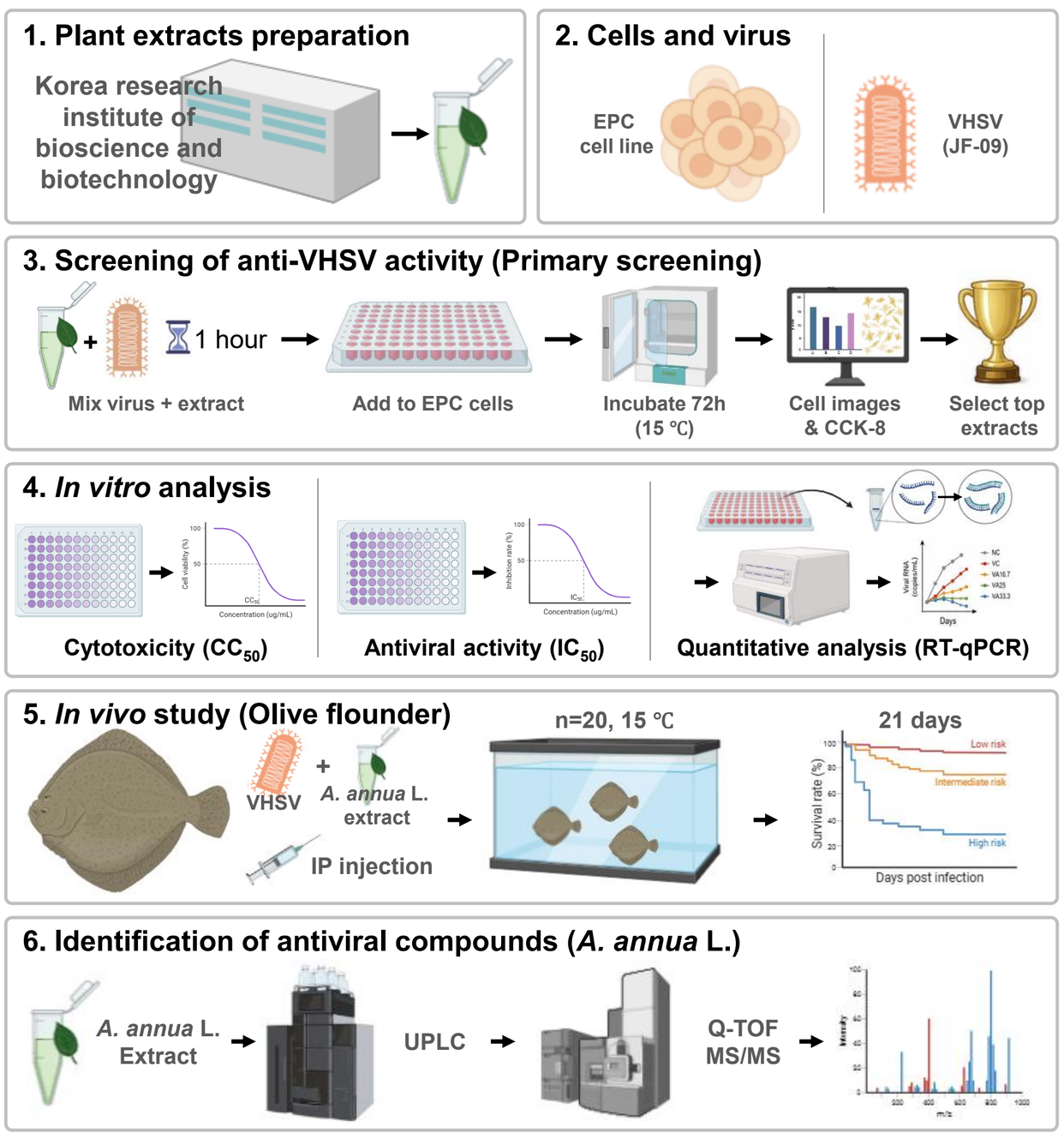
Artemisia annua L., a medicinal plant known for producing the antimalarial compound artemisinin, contains diverse bioactive metabolites with reported antiviral activity (Baggieri et al., 2023). As it is widely used in food and herbal products in Korea, its safety is well established (Trendafilova et al., 2020). This study investigates the potential of *A. annua* L. extract as a natural antiviral agent against VHSV in aquaculture.



Materials and Methods

Table 1. List of natural extracts used this study

No.	Name	Used part
1	*Artemisia annua* L.	aerial part
2	*Eclipta thermalis* Bunge	whole plant
3	*Kadsura japonica* (L.) Dunal	branch
4	*Cedrus deodara (Roxb. ex D.Don) G.Don*	leaf,branch
5	*Rodgersia podophylla* A.Gray	root
6	*Torreya nucifera* (L.) Siebold & Zucc.	leaf
7	*Trichosanthes kirilowii Maxim. var. japonica* (Miq.) Kitam.	whole plant
8	*Paulownia tomentosa* (Thunb.) Steud.	leaf
9	[(*Citrus reticulata* x *Citrus sinensis*) x *Citrus reticulata*] x *Citrus reticulata*	leaf
10	*Nelumbo nucifera* Gaertn.	branch,leaf
11	*Lophatherum gracile* Brongn.	whole plant
12	*Ardisia pusilla* A.DC.	whole plant
13	*Perilla frutescens* (L.) Britton	whole plant
14	*Wollastonia dentata* (H.Lév. & Vaniot) Orchard	whole plant
15	*Broussonetia papyrifera* (L.) L'Hér. ex Vent.	branch,leaf
16	*Poncirus trifoliata* (L.) Raf.	branch
17	*Citrus natsudaidai* Hayata	leaf
18	*Gardenia jasminoides var. radicans* (Thunb.) Makino	leaf
19	*Zanthoxylum piperitum* (L.) DC.	leaf
20	*Euphorbia jolkinii* Boiss.	whole plant
21	*Ophiopogon japonicus* (Thunb.) Ker Gawl.	whole plant
22	*Mirabilis jalapa* L.	whole plant
23	*Pennisetum alopecuroides* (L.) Spreng.	whole plant
24	*Citrus natsudaidai* Hayata	stem
25	*Euphorbia esula* L.	whole plant
26	*Lonicera japonica* Thunb.	leaf,stem
27	*Hibiscus hamabo* Siebold & Zucc.	branch
28	*Citrus natsudaidai* Hayata	bark of a fruit
29	*Fraxinus sieboldiana* Blume	branch,leaf
30	*Fagopyrum esculentum* Moench	whole plant
31	*Bupleurum komarovianum* Lincz.	whole plant
32	*Sigesbeckia orientalis* L.	root
33	*Ranunculus sceleratus* L.	root



Results and discussion

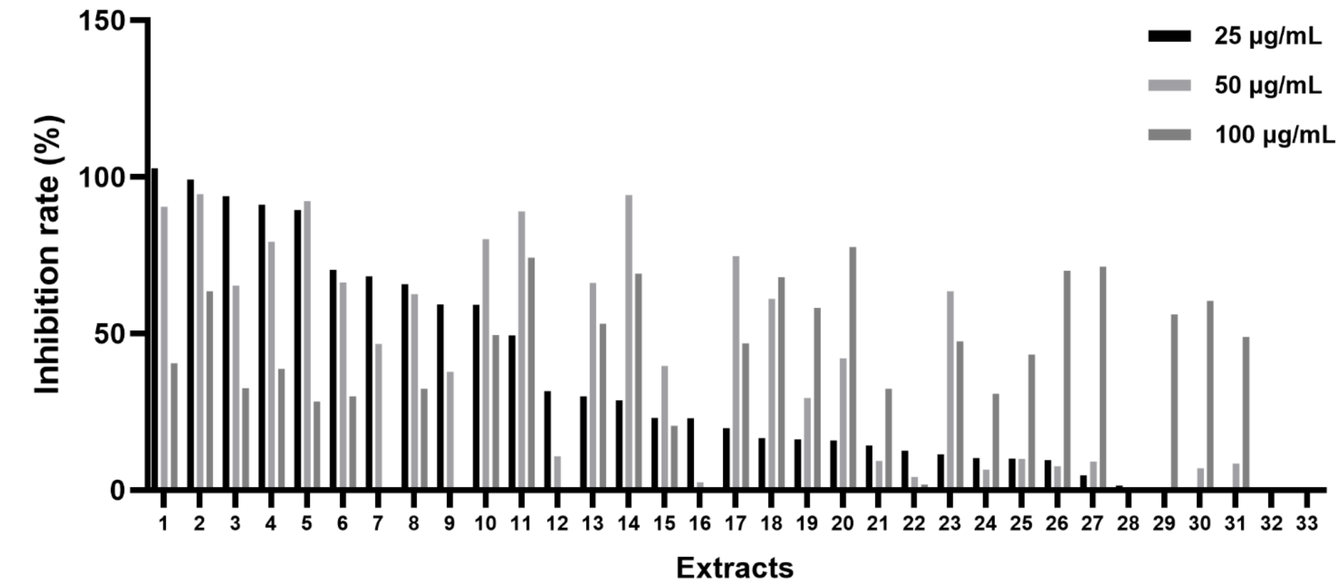


Fig. 1. Screening result against VHSV on EPC cell line. Numbers on the x-axis correspond to plant extracts listed in Table 1.

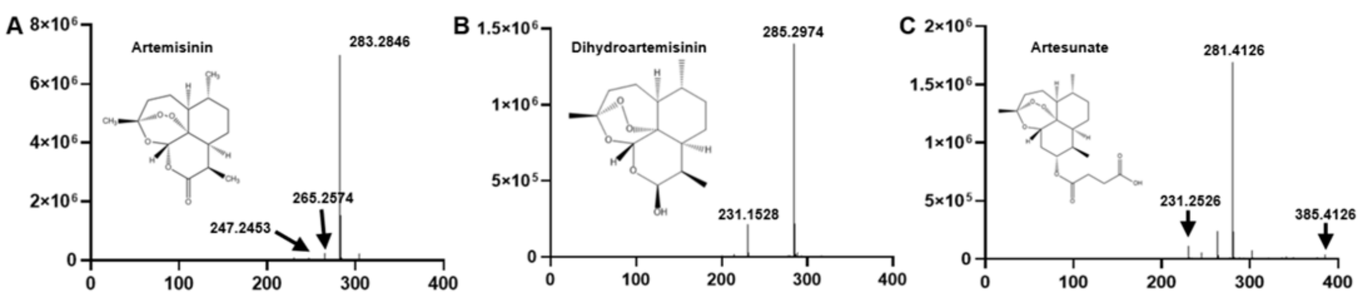


Fig. 2. MS spectrum of antiviral compound derived *A. annua* L. extraction. (A) Artemisinin (m/z 283.2846), (B) Dihydroartemisinin (m/z 285.2974), (C) Artesunate (m/z 385.4126).

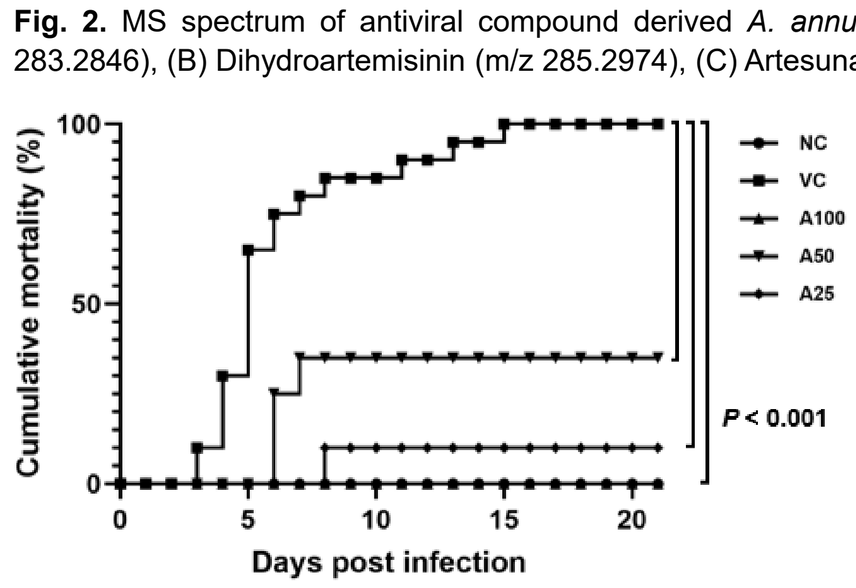


Fig. 3. The cumulative mortality of olive flounder through intraperitoneal injection with VHSV and methanol extract of *A. annua* L..

Abbreviations: NC, Negative control; VC, Virus control; A100, 100 μg/g b.w.; A50, 50 μg/g b.w.; A25, 25 μg/g b.w..

Among the 33 plant extracts screened, *A. annua* L. exhibited the strongest antiviral activity against VHSV and showed the highest selectivity index (SI), indicating high antiviral efficacy with low cytotoxicity. The extract significantly reduced VHSV-induced CPE and improved cell viability in EPC cells in a dose-dependent manner. RT-qPCR analysis further confirmed that viral RNA copy numbers were markedly decreased in extract-treated groups compared to the virus control. In the *in vivo* challenge test, olive flounder treated with *A. annua* L. extract showed improved survival rates following VHSV infection. In addition, UPLC-Q-TOF MS/MS analysis identified several bioactive compounds that may contribute to the antiviral effects of the extract.

Table 2. Toxicity and inhibitory concentrations of the three natural extracts against VHSV in EPC cell line

Name	CC_{50}	IC_{50}	SI
A. annua L.	68.14	< 1.5625	> 43.61
E. thermalis Bunge	90.60	6.91	13.12
K. japonica (L.) Dunal	70.38	10.13	6.95

Table 3. Analysis of viral replication inhibition through quantitative analysis of the cell supernatant

		VHSV N gene Quantity (copies/μL)					
Day	Group	Set 1	Set 2	Set 3	Average	SD	*p*-value
1	NC	ND	ND	ND	0.E+00	0.E+00	0.813
	VC	ND	ND	1.E+00	5.E-01	6.E-01	-
	VA16.7	ND	1.E+00	ND	5.E-01	7.E-01	1
	VA25	ND	ND	ND	0.E+00	0.E+00	0.772
	VA33.3	ND	ND	ND	0.E+00	0.E+00	0.772
2	NC	ND	ND	ND	0.E+00	0.E+00	0.005**
	VC	2.E+01	5.E+00	2.E+01	1.E+01	6.E+00	-
	VA16.7	ND	ND	3.E+00	1.E+00	2.E+00	0.009**
	VA25	ND	ND	ND	0.E+00	0.E+00	0.005**
	VA33.3	ND	ND	ND	0.E+00	0.E+00	0.005**
3	NC	ND	ND	ND	0.E+00	0.E+00	0.033*
	VC	2.E+03	9.E+02	4.E+03	2.E+03	1.E+03	-
	VA16.7	ND	1.E+01	2.E+02	7.E+01	9.E+01	0.039*
	VA25	1.E+01	ND	2.E+00	5.E+00	7.E+00	0.033*
	VA33.3	3.E+00	ND	7.E+00	3.E+00	3.E+00	0.033*
4	NC	ND	ND	ND	0.E+00	0.E+00	0.006**
	VC	6.E+04	2.E+04	3.E+04	4.E+04	2.E+04	-
	VA16.7	5.E+01	3.E+02	3.E+03	1.E+03	1.E+03	0.008**
	VA25	7.E+02	7.E-01	1.E+01	2.E+02	3.E+02	0.007**
	VA33.3	2.E+01	3.E+00	5.E+01	3.E+01	2.E+01	0.006**
5	NC	ND	ND	ND	0.E+00	0.E+00	<.001***
	VC	1.E+05	5.E+04	1.E+05	9.E+04	3.E+04	-
	VA16.7	2.E+03	9.E+00	3.E+04	1.E+04	1.E+04	0.002**
	VA25	4.E+03	2.E+03	3.E+02	2.E+03	2.E+03	0.001**
	VA33.3	4.E+02	4.E+00	9.E+02	4.E+02	4.E+02	<.001***
6	NC	ND	ND	ND	0.E+00	0.E+00	0.237
	VC	1.E+06	1.E+05	1.E+05	4.E+05	4.E+05	-
	VA16.7	2.E+04	3.E+04	9.E+04	5.E+04	3.E+04	0.332
	VA25	2.E+04	2.E+02	1.E+03	8.E+03	1.E+04	0.251
	VA33.3	3.E+03	6.E+01	3.E+03	2.E+03	1.E+03	0.24
7	NC	ND	ND	ND	0.E+00	0.E+00	0.042*
	VC	1.E+06	3.E+05	5.E+05	7.E+05	5.E+05	-
	VA16.7	2.E+05	1.E+05	3.E+05	2.E+05	7.E+04	0.17
	VA25	2.E+05	2.E+03	6.E+04	1.E+05	1.E+05	0.087
	VA33.3	5.E+04	1.E+03	3.E+04	3.E+04	2.E+04	0.051

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