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RESEARCH

# Effect of wogonin, an active ingredient of Scutellariae Radix, on fish infected with *Aeromonas hydrophila*

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## Abstract
Exposure to pathogens induces hyperactivate inflammatory immune responses, oxidative stress and even death in fish. Therefore, suppressing excessive inflammatory responses is a means of treating fish diseases. Scutellariae Radix (SR), an herbal medicine, has anti-inflammatory and antioxidant properties. However, the active ingredients of SR that produce a marked effect and their mechanisms are poorly understood. In this study, 32 active ingredients of SR and their 124 target genes associated with anti-inflammatory effect were screened. These target genes in zebrafish were mainly enriched in biological processes associated with response to stress and stimulus. The KEGG enrichment analysis and pathway–pathway network analysis showed that the MAPK signalling pathway and nine other pathways were the key pathways associated with anti-inflammatory effect. A protein–protein interactions (PPI) network of target genes was constructed, and **AKT2** and nine other genes were identified as the hub genes by cytoHubba. Combining the above results, we constructed an active ingredient–hub protein–key pathway network, showing that wogonin-AKT2 was the key regulated module. Molecular docking and SPR-based assays were used to investigate the possible interaction of wogonin with the **AKT2** protein. The infected zebrafish and grass carp treated with wogonin displayed a higher survival rate and anti-inflammatory and antioxidant effects than the infected fish without wogonin treatment. Dietary wogonin also enhanced grass carp anti-inflammatory and antioxidant effects. These findings further reveal the molecular biological mechanism by which wogonin from SR exerts anti-inflammatory effects and provide a theoretical basis for the prevention of fish diseases.

**Keywords** Wogonin · Inflammatory · Scutellariae radix · Fish · AKT2

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## Introduction

In recent years, with the expansion of artificial cultivation, fish diseases caused by various pathogens have frequently occurred. This higher disease rate has hindered the development of fish culture and led to major economic losses. When pathogens infect a host, inflammation occurs to protect against tissue damage. However, some pathogens induce hyperactivation of the immune system, leading to exacerbated inflammatory responses, oxidative stress, cytokine storms, organ failure, tissue damage and even death (Oh and Lee 2023). For example, *Aeromonas hydrophila* is one of the fish pathogens responsible for bacterial sepsis, an inflammatory reaction that is induced by pathogenic bacteria infiltrating the bloodstream and releasing toxins (Niu et al. 2023). Therefore, suppressing excessive inflammatory responses may be a strategy to treat fish diseases.

Natural products as feeding additives have been used to enhance fish growth and immunity. For example, oregano (*Origanum vulgare*) extracts support the innate immune response of zebrafish (*Danio rerio*) in fighting against bacterial infection (Rashidian et al. 2021; Xia et al. 2022). Moreover, other natural products also play an important role in the anti-inflammatory response. The essential oil from the leaves of *Psidium cattleyanum* has anti-inflammatory effects on mice with carrageenan-induced paw oedema and peritonitis (Bruna Guimarães Silva et al. 2023). Natural products also affect the fish anti-inflammatory response. Rice bran oil extract has been shown to have anti-inflammatory and antioxidant properties in copper sulphate–induced inflammation in zebrafish (Liu et al. 2023a, b). Supplemental octanoate relieves oxidative stress and regulates the inflammatory response in large yellow croaker fed a high soybean oil diet (Zhao et al. 2023).

Scutellariae Radix (SR, Huang Qin in Chinese), the dried root of the medicinal plant *Scutellariae baicalensis* Georgi, contains multiple natural products and is used to treat several diseases due to its anti-inflammatory and antioxidant properties (Cho et al. 2013). For example, a previous study has showed that SR is used in traditional Chinese medicine for treating lung diseases due to its remarkable anti-inflammatory and antiviral effects (Zhang et al. 2021). Moreover, in a previous study on fish, SR also exerts anti-inflammatory effects on rabbit fish (*Siganus fuscescens*) and inhibits the expression of Lipopolysaccharide (LPS)-induced inflammatory cytokines in rabbit fish macrophages (Xia et al. 2021). In addition, SR exerts anti-hypoxia effects on cultured fin cells from rabbit fish by preventing hypoxia-induced signalling (Xia et al. 2020). After olive flounder (*Paralichthys olivaceus*) is fed *S. baicalensis* extract, the mortality of fish during *Edwardsiella tarda* infection was mitigated (Cho et al. 2013). In addition, SR also enhances fish immune activity. For example, the immune activity of pearl gentian grouper (a hybrid of *Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) is enhanced after treatment with *S. baicalensis* extract, as exhibited by the upregulated expression of anti-inflammatory cytokines (Xia et al. 2022). Although *S. baicalensis* is involved in the fish anti-inflammatory response, the active ingredients that produce a marked effect and their regulatory network are poorly understood.

In this study, we first obtained the active ingredients of SR from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and identified their target genes in zebrafish. Next, a protein−protein interaction (PPI) network of these target genes was constructed, and the cytoHubba plugin was used to identify the hub genes. Combining GO and KEGG results, we identified the key active ingredient-target gene module. Third, molecular docking and SPR-based assays were used to investigate the possible interaction of the key active ingredient with the target protein. Finally, the key active ingredient was used to treat the infected zebrafish and grass carp, and feed grass carp as additives, and the antioxidant and anti-inflammatory properties of the active ingredient were examined by evaluating the total antioxidant capacity, inflammatory gene levels and survival rate of fish.

## Materials and methods

### SR active ingredient database

The ingredients of SR were downloaded from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/index.php), which includes their pharmacokinetic properties, such as oral bioavailability (OB) and drug-likeness (DL). Based on these, active ingredients were obtained with OB $\geq$ 30% and DL $\geq$ 0.18 as the screening conditions, and the active ingredient MOL2 files were also downloaded for the preparation of molecular docking.

### Construction and analysis of PPI network of target genes of the SR active ingredient in zebrafish

A protein−protein interaction (PPI) network was constructed to evaluate the gene interactions between the potential target genes. The target protein sequences were inputted to the STRING database version 11.5 to construct a PPI network with '*Danio rerio*' being selected. The PPI network was submitted to Cytoscape_3.9.1, and the cytoHubba plugin was used to identify the hub genes of this network. GO and KEGG analyses were also performed using the STRING database. All protein sequences were entered in the 'SEARCH' column, species was selected as *Danio rerio* and then analysed. In the 'Analysis' column of the results, the GO and KEGG analysis results were downloaded.

### Docking calculation

The active ingredients acting on the hub proteins were used as the ligands for the molecular docking study. The three-dimensional (3D) structures of the proteins coded by hub genes were simulated by AlphaFold 2.3.2 and displayed by PyMOL 2.1.0. Virtual screening of active ingredients against hub proteins was carried out using CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/index.php). The binding poses with the lowest Vina score for the molecular docking models were collected for visualising docking structures.

### Surface plasmon resonance assay

Surface plasmon resonance (SPR) analysis was conducted with an Open SPR instrument (Nicoyalife, Canada). The receptor protein was loaded onto the COOH sensor chip. The ligand compound was diluted into a series of solutions with different concentrations, which were then injected into the chip from low to high concentrations. In each cycle, the sample (200 μL) flowed through the chip for 7 min at a constant flow rate of 20 μL/min. The detailed operation was performed according to the standard procedure of OpenSPR. Trace Drawer software was used to calculate the kinetic parameters of the binding reactions.

### *A. hydrophila* challenge of zebrafish and grass carp and treatment with wogonin

Zebrafish (approximately 0.6 g in weight and 12 months old) and grass carp *Ctenopharyngodon idella* (approximately 15 g in weight and 1 month old) were used for experiments at the State Key Laboratory of Developmental Biology of Freshwater Fish after phenotype observation and virus testing. Zebrafish were raised at 28.5 °C in the automated circulatory system, and grass carp were maintained in aerated freshwater at 25 ± 1 °C. The two types of fish were divided into three groups: the control group, infection group and treatment group (3 replicates of 20 fish per group). *A. hydrophila* stored in our laboratory was picked and inoculated into LB plate at 30℃ for 24 h. The single clone was picked and incubated in liquid LB medium at 30℃ for 24 h with shaking at 180 r/min. The liquid medium was centrifuged at 3000 × *g* for 10 min to remove residual culture medium, and the pellets containing bacterial cells were washed twice with phosphate-buffered saline (PBS, pH 7.4). The final bacterial pellet was resuspended in PBS, and the solution's absorbance was adjusted to 0.5 at 600 nm, corresponding to $1 \times 10^7$ CFU/mL. Wogonin was acquired from Aladdin Biology Technology Institute (W101155, CAS 632–85-9, Shanghai, China). The *A. hydrophila* infection model in zebrafish was established by intraperitoneal injection of zebrafish with $1 \times 10^7$ CFU/mL *A. hydrophila* (10 μL) in the infection group and treatment group. The control group was injected with 10 μL PBS. The grass carp in the infection group and treatment group were intraperitoneally injected with 200 μL of *A. hydrophila* at a concentration of $1 \times 10^7$ CFU/mL. The control group was injected with 200 μL PBS. At 2 days post-infection, the fish in the treatment group were injected with 10 and 200 μL wogonin solution (2 mg/mL saline solution) and maintained in aerated freshwater until 7 days. Whole zebrafish and liver and blood samples of grass carp were harvested for antioxidant activity evaluations and RNA extraction to examine the expression levels of pro- and anti-inflammatory genes by RT-qPCR.

### Feeding grass carp with dietary wogonin

After phenotype observation and virus testing, healthy grass carp of both sexes, approximately 4 months old and weighing approximately 50 g, were provided by the State Key Laboratory of Developmental Biology of Freshwater Fish. Grass carp were randomly allocated into six tanks (1 × 2 × 2 m) and maintained in aerated freshwater at 25 ± 1 °C for 3 days. The fish were divided into two groups: the control group and the treatment group (3 replicates of 50 fish per group). The rearing conditions were water temperature at 25 ± 1 °C, pH 7–8, dissolved oxygen content 7 mg/L and ammonia nitrogen content < 0.02 mg/L. The rearing experiment was carried out in a flow-through aquaculture system with 60% water changes once a day. During this experiment, the grass carp were fed daily at 8:30 and 16:30 and the daily feeding amount was 6% of the fish weight. The basal diet was supplemented with wogonin (20 mg/kg) (Table S1). Dietary ingredients were crushed and mixed well through a 40-mesh sieve and then processed into feeds with a diameter of 2.0 mm by using an SLX-80 extruder. The diets were dried and cooled at 45 °C, and then placed in a sealed bag and stored in a refrigerator at − 15 °C. The control group was fed the basal diet and the wogonin group was fed the basal diet supplemented with wogonin. Fish were weighed once a week for a 6-week experimental period. The grass carp in the control group and dietary wogonin group were then injected intraperitoneally with 1000 μL of *A. hydrophila* at a concentration of $1 \times 10^7$ CFU/mL. Fish mortality was recorded daily. We determined whether the death was caused by *A. hydrophila* by observing whether the fish exhibited the typical symptoms of *A. hydrophila*: congestion on the body surface, enlarged liver and spleen, congested intestinal wall, and red, swollen and protruding anus. At 7 days post-infection, the liver and blood samples from grass carp were collected for antioxidant activity evaluations and RNA extraction to examine the expression levels of pro- and anti-inflammatory genes by RT-qPCR.

### Measurements of T-AOC, SOD and CAT activities

The total antioxidant capacity (T-AOC) and SOD and CAT activities were measured with commercial kits from Nanjing Jiancheng Institute (Nanjing, China) according to the manufacturer's instructions.

### RT-qPCR analysis

All RT-qPCRs were carried out with three independent biological replicates. RT-qPCR was performed with a SYBR Premix Ex TaqTM II Kit (TaKaRa) according to the manufacturer's protocol. The reaction was carried out on an ABI7500 platform. Information on all primers is shown in Table S2. The Ct values of each gene were standardised, and the relative changes for each gene were analysed by the $2^{-\Delta\Delta Ct}$ method, where $\Delta CT = CT_{gene} - CT_{actin}$ and $\Delta\Delta CT = \Delta CT_{test\ group} - \Delta CT_{control\ group}$. The formula includes the following mathematical expressions: $\Delta\Delta CT = \Delta CT1 - \Delta CT2$, where $\Delta CT1 = CT$ of the examined gene (experimental group) – CT of the reference control gene (experimental group) and $\Delta CT2 = CT$ of the examined gene (control group) – CT of the reference control gene (control group). The final result is the calculation of $2^{-\Delta\Delta CT}$.

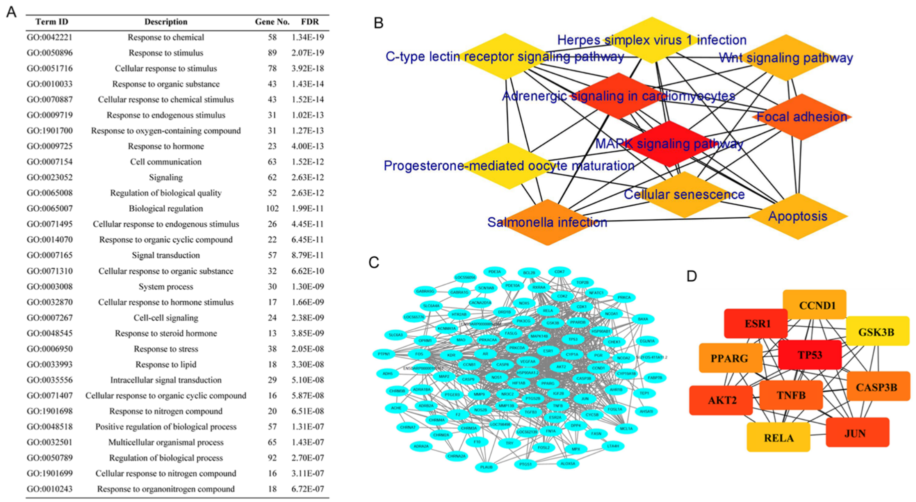
## Results

### SR active ingredients and their target genes

A total of 143 active ingredients from SR were obtained from the TCMSP database (Table S3). Among these active ingredients, 36 were found with OB ≥ 30% and DL ≥ 0.18, such as wogonin, baicalein and acacetin. From the TCMSP database, it was found that 32 active ingredients could target 124 genes (Table S4).

### GO and KEGG pathway enrichment analysis

After BLASTp searches against zebrafish genome data (GRCz10), the target genes of the 32 active ingredients were identified in zebrafish. These genes were subjected to GO enrichment analysis. The distribution of the genes in different GO categories is shown in Table S5. One or more genes were enriched in 218 biological process terms, 73 molecular function terms and 24 cellular component terms. The top 30 GO terms in the biological process category were associated with responses to various stresses and stimuli (Fig. 1A). The biological processes of these target genes mainly included response to chemical stimulus (GO:0042221), response to stimulus (GO:0050896), cellular response to stimulus




| Term ID | Description | Gene No. | FDR |
|---|---|---|---|
| GO:0042221 | Response to chemical | 58 | 1.34E-19 |
| GO:0050896 | Response to stimulus | 89 | 2.07E-19 |
| GO:0051716 | Cellular response to stimulus | 78 | 3.92E-18 |
| GO:0010033 | Response to organic substance | 43 | 1.43E-14 |
| GO:0070887 | Cellular response to chemical stimulus | 43 | 1.52E-14 |
| GO:0009719 | Response to endogenous stimulus | 31 | 1.02E-13 |
| GO:1901700 | Response to oxygen-containing compound | 31 | 1.27E-13 |
| GO:0009725 | Response to hormone | 23 | 4.00E-13 |
| GO:0007154 | Cell communication | 63 | 1.52E-12 |
| GO:0023052 | Signaling | 62 | 2.63E-12 |
| GO:0065008 | Regulation of biological quality | 52 | 2.63E-12 |
| GO:0065007 | Biological regulation | 102 | 1.99E-11 |
| GO:0071495 | Cellular response to endogenous stimulus | 26 | 4.45E-11 |
| GO:0014070 | Response to organic cyclic compound | 22 | 6.45E-11 |
| GO:0007165 | Signal transduction | 57 | 8.79E-11 |
| GO:0071310 | Cellular response to organic substance | 32 | 6.62E-10 |
| GO:0003008 | System process | 30 | 1.30E-09 |
| GO:0032870 | Cellular response to hormone stimulus | 17 | 1.66E-09 |
| GO:0007267 | Cell-cell signaling | 24 | 2.38E-09 |
| GO:0048545 | Response to steroid hormone | 13 | 3.85E-09 |
| GO:0006950 | Response to stress | 38 | 2.05E-08 |
| GO:0033993 | Response to lipid | 18 | 3.30E-08 |
| GO:0035556 | Intracellular signal transduction | 29 | 5.10E-08 |
| GO:0071407 | Cellular response to organic cyclic compound | 16 | 5.87E-08 |
| GO:1901698 | Response to nitrogen compound | 20 | 6.51E-08 |
| GO:0048518 | Positive regulation of biological process | 57 | 1.31E-07 |
| GO:0032501 | Multicellular organismal process | 65 | 1.43E-07 |
| GO:0050789 | Regulation of biological process | 92 | 2.70E-07 |
| GO:1901699 | Cellular response to nitrogen compound | 16 | 3.11E-07 |
| GO:0010243 | Response to organonitrogen compound | 18 | 6.72E-07 |

**Fig. 1** Functional prediction of potential target genes. **A** The top 30 remarkably enriched GO terms in biological progress. **B** The key KEGG pathways of the target genes. These pathways were identified using the cytoHubba plugin in the pathway–pathway relationship network. **C** The PPI network of potential target genes using the STRING database. **D** The hub target genes of the PPI network. The hub genes were identified using the cytoHubba plugin of Cytoscape

(GO:0051716), response to organic substance (GO:0010033) and cellular response to chemical stimulus (GO:0070887).

To deepen our understanding of the mechanism of action of SR, KEGG pathway enrichment analysis of the target genes was also performed. A total of 39 pathways were obtained from KEGG pathway enrichment analysis ($p < 0.05$). These target genes were mainly involved in the p53 signalling pathway, apoptosis, the MAPK signalling pathway, the calcium signalling pathway and others (Table S6). Among these KEGG pathways, it was found that the target genes were assigned to more than one pathway. As a result, a pathway–pathway relationship network was constructed by Cytoscape (Fig. S1). Using the cytoHubba plugin in Cytoscape, the top 10 pathways were identified with node scores as key KEGG pathways (Fig. 1B). The darker the colour of the pathway is, the higher the ranking is. These key KEGG pathways were the MAPK signalling pathway, adrenergic signalling in cardiomyocytes, focal adhesion, *Salmonella* infection and others.

## PPI network of target genes in zebrafish

A PPI network map is beneficial for gene function analysis, gene interaction prediction and key gene identification. Sequentially, these target genes were imported into the STRING database to obtain the PPI network map. The PPI enrichment $p$ value was $< 1.0e-16$, the number of nodes was 115 and the number of lines was 730 in the PPI network (Fig. 1C).

Using the cytoHubba plugin in Cytoscape, the top 10 nodes were identified with node scores from the PPI network, which were the hub genes of this network. As shown in Fig. 1D, the darker the colour of the network graph gene is, the higher the ranking is. These 10 hub genes were cellular tumour antigen p53 (**TP53**), oestrogen receptor alpha

(**ERS1**), AP-1 transcription factor (**JUN**), v-akt murine thymoma viral oncogene homologue (**AKT2**) and others.

## Key active ingredient-target gene module

We constructed an active ingredient–hub protein–key pathway network, displaying that 13 active ingredients (Table S7) acted on 10 hub proteins. Of these, only wogonin could act on all hub proteins (Fig. 2A, Table S8). Of the 10 hub proteins, **AKT2** (ENSDARP00000023119) was involved in the most pathways, affecting 9 key pathways except the Wnt signalling pathway. Overall, wogonin-AKT2 was selected as the key regulated module.

To clarify the interaction between wogonin and hub proteins, molecular docking was performed. The 3D structures of the hub proteins were predicted by AlphaFold, and the results are shown in Fig. S2. Wogonin, 5,7-dihydroxy-8-methoxyflavone, is a flavonoid-like chemical compound (Table S7). During molecular docking between wogonin and hub proteins, the lower the Vina scores are, the more stable the binding of wogonin to the hub proteins is, and these scores were used to preliminarily evaluate the binding activity of wogonin to the hub proteins. CB-DOCK2 was used for molecular docking, and the docking coefficient, size and lowest Vina score are shown in Table S9. If their Vina score are less than − 7.0 kcal/mol, both indicate a high binding activity. The Vina scores of wogonin with **AKT2**, **CCND1**, GSK3B, **PPARG**, **TP53** and **ESR1** were all less than − 7.0 kcal/mol, indicating that wogonin has a high binding activity with them. The docking results between wogonin and each hub protein are shown in Fig. 2B and Fig. S3. The docking results between wogonin and **AKT2** proteins showed that four hydrogen bonds were formed between wogonin and ASP291 and LYS179 in the akt2 protein. In addition, wogonin with

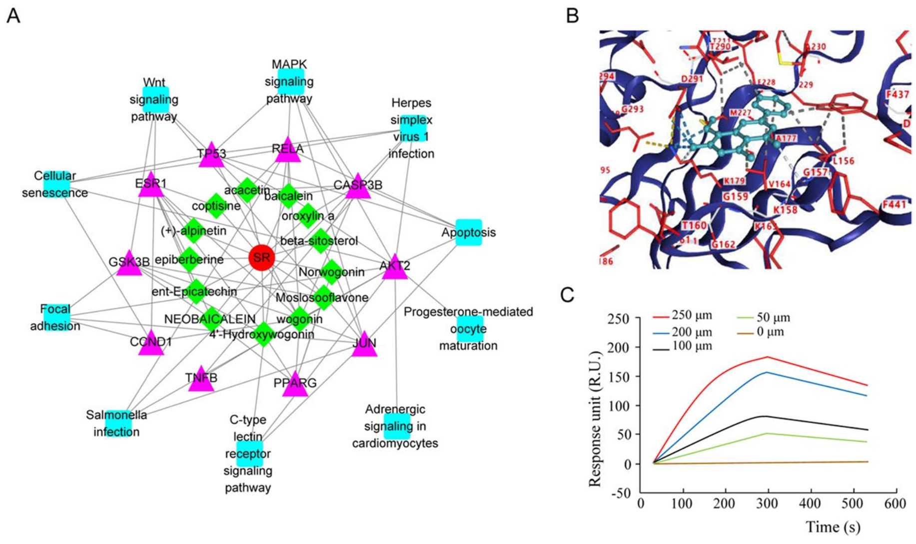



**Fig. 2** Interactions between wogonin and the **AKT2** protein. **A** SR-active ingredient–hub target–key KEGG pathway network. **B** The binding pattern between wogonin and the **AKT2** protein in the molecular docking model. **C** SPR assay showing the interactions between wogonin and the **AKT2** protein

LYS179 and GLY157 formed two weak hydrogen bonds and six hydrophobic contacts with VAL164, ALA177, LEU156, PHE437 and THR290 in the **AKT2** protein.

The interaction of wogonin and **AKT2** was further evaluated by real-time biomolecular interaction analysis with SPR. The kinetics of the binding reaction were determined by injecting different concentrations of wogonin over a recombinant **AKT2** protein immobilised on the chip surface. The data were fitted to a monovalent binding model by nonlinear regression, and the equilibrium dissociation constant ($K_D$) for wogonin was 2.14e − 5 (Fig. 2C).

## Wogonin positively contributes to anti-inflammation and antioxidant after pathogen infection

Inflammation in fish is partially related to cytokines, with inflammation driven by pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) increase inflammation, while anti-inflammatory cytokines (IL-10) decrease inflammation (Niu et al. 2023). After infection with *A. hydrophila*, the expression levels of pro-inflammatory genes, including *TNF-α*, *IL-6* and *IL-1β*, were significantly increased in zebrafish compared with the control group (Fig. 3A). Seven days post-treatment with wogonin, compared with the infection group, the pro-inflammatory gene levels were significantly decreased in the treatment group, and the anti-inflammatory gene (*IL-10*) level was significantly increased (Fig. 3B). Moreover, the activities of SOD and CAT and T-AOC were higher in the treatment group than in the infection group (Fig. 3C). In addition, the survival rate of the treatment group at 7 days post-infection was 46.7%, which was significantly higher than that of the infection group (16.7%) (Fig. 3D).

Moreover, the economic fish grass carp was also infected with *A. hydrophila.* The *TNF-α*, *IL-6* and *IL-1β* levels were significantly increased in the liver and blood samples of the infected grass carp compared with those in the liver and blood samples of the control fish (Fig. 3E). Seven days post-treatment with wogonin, compared with the infection group, the *TNF-α*, *IL-6* and *IL-1β* levels were significantly decreased in the treatment group, and the *IL-10* level was significantly increased (Fig. 3F). The antioxidant capacity was enhanced (Fig. 3G), and the survival rate was significantly increased compared with that of the infection group (Fig. 3H).

These results suggested that wogonin may enhance the anti-inflammatory and antioxidant capacity of fish, thus increasing the fish survival rate after infection with pathogens.

## Dietary wogonin enhances grass carp resistance to *A. hydrophila.*

To clarify the effect of dietary wogonin, after 14 days of acclimation, grass carp were fed a basal diet supplemented with wogonin for 42 days. Then, the grass carp were challenged with *A. hydrophila*, and 7 days post-treatment, their resistance to *A. hydrophila* was examined (Fig. 4A). The pro-inflammatory- and anti-inflammatory-related gene levels were also examined. Compared to that in the fish fed the basal diet (control group), the expression level of the anti-inflammatory-related gene *IL-10* was significantly increased in both liver and blood samples of the fish fed the basal diet supplemented with wogonin, while the pro-inflammatory-related genes *TNF-α*, *IL-6* and *IL-1β* were inhibited (Fig. 4B). These results suggested that dietary wogonin may enhance fish anti-inflammatory activity.

To detect the disease resistance of fish after feeding the basal diet supplemented with wogonin, an *A. hydrophila* challenge experiment was performed. After a 7-day infection

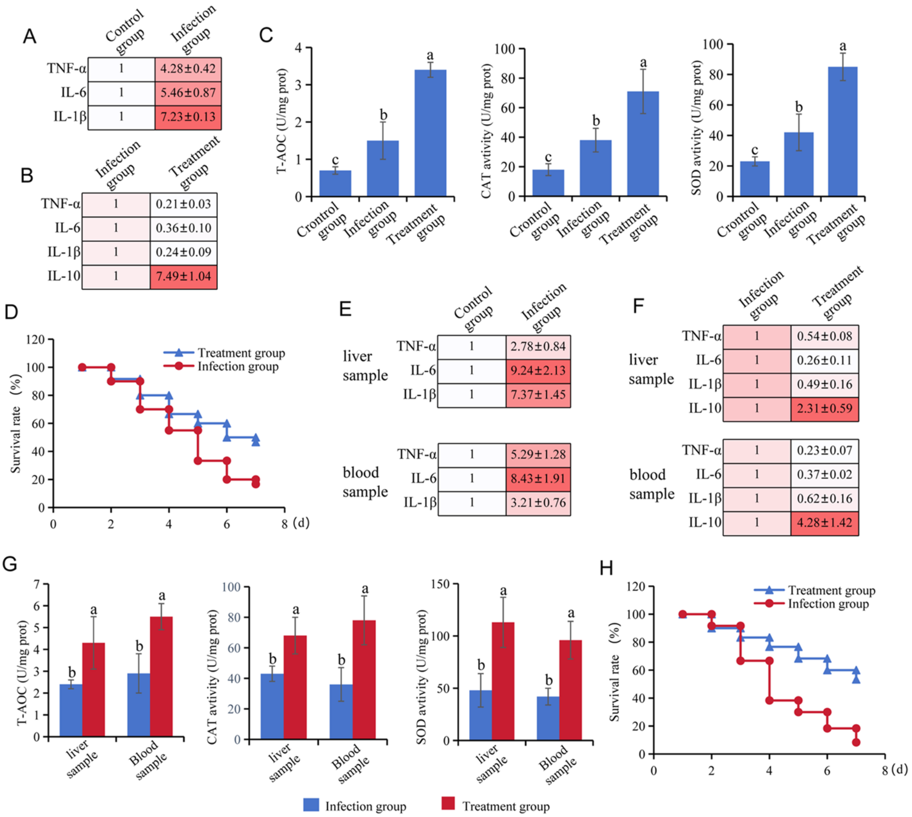



**Fig. 3** Wogonin positively contributes to anti-inflammation and antioxidant after pathogen infection. **A** Expression levels of pro-inflammatory genes (*TNF-α*, *IL-6*, *IL-1β*) in zebrafish infected with *A. hydrophila*. **B** Expression levels of pro- and anti-inflammatory genes in the treatment group of zebrafish at 7 days. **C** T-AOC and activities of SOD and CAT in the treatment group of zebrafish at 7 days. **D** Cumulative survival of zebrafish treated with wogonin after infection with *A. hydrophila*. **E** Expression levels of *TNF-α*, *IL-6* and *IL-1β* genes in the liver and blood samples of grass carp infected with *A. hydrophila*. **F** Expression levels of *TNF-α*, *IL-6*, *IL-1β* and *IL-10* genes in the liver and blood samples of the treatment group of grass carp at 7 days. **G** T-AOC and activities of SOD and CAT in the liver and blood samples of the treatment group of grass carp at 7 days. **H** Cumulative survival rate of grass carp treated with wogonin after infection with *A. hydrophila.* Error bar, mean ± SD. Letters indicate significant differences among samples ($p < 0.05$). Grass carp *Actin* was used as a reference control gene for RT-qPCR analysis

with *A. hydrophila*, the abundance of *A. hydrophila* was also examined in the liver and blood samples through the transcript level of *A. hydrophila GAPDH.* The results revealed significant increases in the abundance of *A. hydrophila* in the liver and blood samples of the fish fed the basal diet compared to that in the fish fed the basal diet supplemented with wogonin (Fig. 4C). In addition, the fish fed the basal diet supplemented with wogonin exhibited a higher survival rate than the fish fed the basal diet (Fig. 4D). After infection with *A. hydrophila*, the fish were fed the basal diet supplemented with wogonin displayed an increase in SOD and CAT activities and T-AOC (Fig. 4E), suggesting that wogonin may enhance fish antioxidant capacity. These results suggested that the dietary wogonin enhances grass carp resistance to *A. hydrophila* by increasing antioxidant capacity and anti-inflammatory activity.

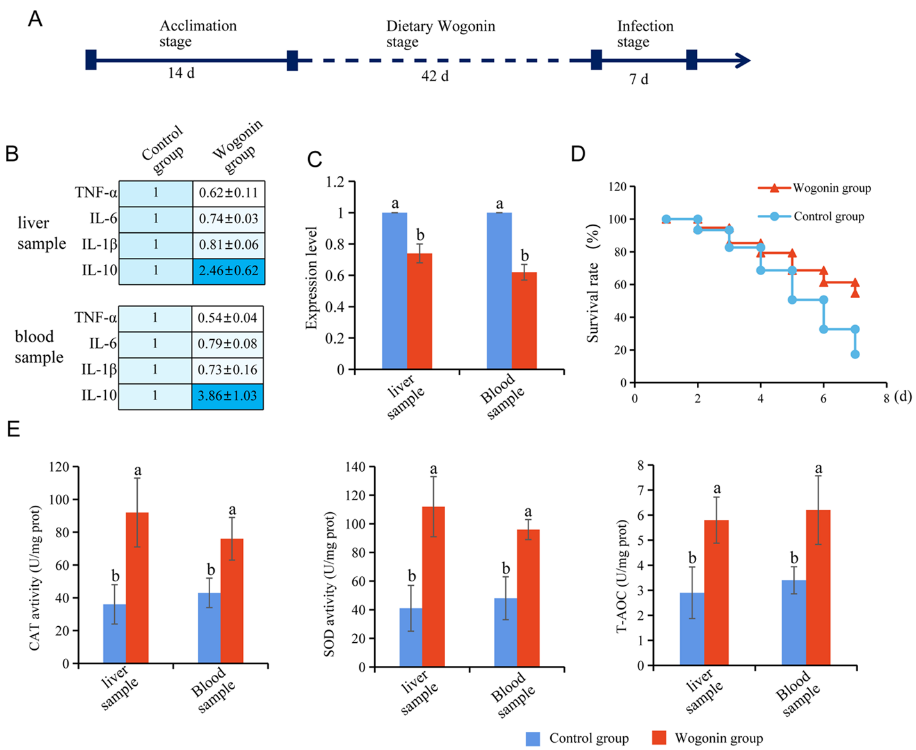



**Fig. 4** Dietary wogonin enhances the anti-inflammatory, antioxidant and resistance of grass carp to *A. hydrophila*. **A** Schematic showing the experimental procedure for the grass carp infected with *A. hydrophila* after feeding with a basal diet supplemented with wogonin. **B** Expression levels of pro-inflammatory (*TNF-α*, *IL-6*, *IL-1β*) and anti-inflammatory (*IL-10*) genes in the liver and blood samples of the grass carp fed a basal diet supplemented with wogonin. **C** Accumulation of the transcript of *A. hydrophila GAPDH* in the blood sample of grass carp infected with *A. hydrophila* after feeding with the basal diet supplemented with wogonin. **D** Cumulative survival rate of grass carp infected with *A. hydrophila* after feeding with the basal diet supplemented with wogonin. **E** T-AOC, SOD and CAT activity in the liver and blood samples of the grass carp fed with the basal diet supplemented with wogonin after infection with *A. hydrophila*. Error bar, mean ± SD. Letters indicate significant differences among samples ($p < 0.05$). Grass carp *Actin* was used as a reference control gene for RT-qPCR analysis

## Discussion

*A. hydrophila*, a gram-negative bacterium, is a pathogen affecting the aquaculture industry (Yun et al. 2020). Diseases caused by *A. hydrophila* in freshwater fish species result in millions of dollars of economic losses worldwide (Dong et al. 2021). This pathogen induces an excessive inflammatory response and oxidative stress by secreting of virulence factors, such as adhesins, cytotoxins and hemolysins (Niu et al. 2023; Yun et al. 2020). When *A. hydrophila* invades fish, ROS levels increase, and inflammation-related signalling pathways are promoted (Gu et al. 2022). Therefore, the treatment and prevention of the disease caused by *A. hydrophila* can be achieved by increasing the anti-inflammatory activity and reducing the oxidative effects in fish.

Several natural substances have been used to prevent *A. hydrophila* infection by enhancing anti-inflammatory activity and reducing oxidative effects. For example, koumine could affect the expression of antioxidant and immune genes in various tissues and significantly improve the antioxidant and immune abilities of carp (*Cyprinus carpio*), which is conducive to improving the resilience of carp to *A. hydrophila* (Wang et al. 2024). Dietary coriander (*Coriandrum sativum* L.) oil improves antioxidant and anti-inflammatory activity, innate immune responses and resistance to *A. hydrophila* in Nile tilapia (*Oreochromis niloticus*) (Das et al. 2023). SR has multiple pharmacological effects, particularly anti-inflammatory and antibacterial (Luo et al. 2024; Liao et al. 2021). SR compounds exert anti-inflammatory, antibacterial, antiviral and immunomodulatory effects by inhibiting inflammation-related pathways or targeting pathogens (Liao et al. 2021).

Wogonin, a flavonoid-like chemical compound from SR, has anti-inflammatory and antioxidant effects (Fan et al. 2020). In addition, inflammation can lead to excessive production of ROS, contributing to the imbalance between pro- and antioxidants and causing oxidative stress (Kadioglu et al. 2015). Thus, inflammatory responses and oxidative stress occur simultaneously. A previous study has shown that wogonin inhibits the production of inflammatory cytokines and has antioxidant properties in pathogenic bacteria–induced inflammatory responses (Yang et al. 2024). The optimal anti-inflammatory effects of wogonin were successfully validated in the zebrafish model (Sun et al. 2023). In addition, wogonin inhibits pathogen infection. For example, wogonin inhibits latent HIV-1 reactivation by downregulating histone crotonylation (Zhang et al. 2023). Moreover, wogonin exhibits antiviral activity against a porcine epidemic diarrhoea virus (PEDV) variant isolate, interacting with PEDV particles and inhibiting the internalisation, replication and release of PEDV (Wang et al. 2023). In the study on fish disease, wogonin had the strongest antibacterial activity against the bacteria *Edwardsiella ictaluri* and *Flavobacterium columnare*, which cause enteric septicaemia and columnaris disease, respectively, in channel catfish (*Ictalurus punctatus*) (Schrader 2010). In this study, we used a network pharmacology method to predict that wogonin, a key active ingredient for SR, contributes to anti-inflammation. After zebrafish and grass carp infected with *A. hydrophila* were treated with wogonin, inflammation and oxidative stress were inhibited, leading to a markedly increased survival rate of grass carp infected with *A. hydrophila*. Meanwhile, after infection with *A. hydrophila*, the grass carp fed a basal diet supplemented with wogonin exhibited much stronger anti-inflammatory and antioxidant effects, as shown by reduced ROS levels, increased anti-inflammatory gene expression and increased survival rates. These results suggest that wogonin positively contributes to grass carp resistance to *A. hydrophila* by reducing inflammation and oxidative stress.

The AKT protein is a key regulatory factor that is involved in various pathways, such as the MAPK signalling pathway, VEGF signalling pathway, cell survival and apoptosis. Moreover, in this study, it was also found that **AKT2** can affect 9 key pathways (10 in total) in zebrafish, suggesting that **AKT2**, a target of SR active ingredients, may play the most critical regulatory role during pathogen infection. The AKT pathway was inhibited to activate the anti-inflammatory response. For example, chrysin can inhibit the PI3K/AKT/mTOR signalling pathway to decrease the synthesis and release of pro-inflammatory cytokines and inflammatory mediators and thus produce anti-inflammatory and antioxidant effects (Cai et al. 2012). Moreover, wogonin also inhibits the AKT pathway. A previous study showed that wogonin increases gemcitabine sensitivity in pancreatic cancer by inhibiting the AKT pathway (Zhang et al. 2022). Wogonin significantly attenuated airway resistance and lung inflammation by decreasing the levels of inflammatory cytokines and key factors in the PI3K/AKT, IL-17 and TNF signalling pathways (Liu et al. 2023a, b). In addition, wogonin is used to alleviate kidney tubular epithelial injury in patients with diabetic nephropathy. This treatment is used because wogonin affects the PI3K/Akt/NF-κB signalling pathway to downregulate the expression of pro-inflammatory cytokines and thus regulate autophagy and inflammation (Lei et al. 2021). In this study, through molecular docking and SPR-based assays, it was found that wogonin decreased **AKT2** protein levels in zebrafish. After wogonin-treated zebrafish and grass carp were infected with *A. hydrophila*, the inflammatory response of both zebrafish and grass carp was suppressed, displaying a decrease in the levels of pro-inflammatory cytokines, an increase in the levels of anti-inflammatory cytokines and enhanced antioxidant activities, thus leading to increased survival rates of zebrafish and grass carp.

## Conclusion

The biological functions and signalling pathways of the SR active ingredients were investigated by a network pharmacology approach. The MAPK signalling pathway and nine other pathways were key pathways. Among these pathways, the **AKT2** protein was located in the control centre as a key factor. Wogonin, an anti-inflammatory compound from SR, interacted with the **AKT2** protein according to the molecular docking and SPR-based assays. Wogonin enhanced the anti-inflammatory and antioxidant capacity of fish, thus increasing the fish survival rate after infection with pathogens. These findings further reveal the molecular biological mechanism of wogonin and SR in the anti-inflammatory response and provide a theoretical basis for the treatment of fish diseases.

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**Author contribution** Jun Cui designed, managed and conducted the experiments, and wrote the manuscript. Dan Zeng performed network pharmacology analysis. Peipei Guan analyzed data.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

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