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RECEIVED 20 April 2025

ACCEPTED 23 June 2025

PUBLISHED 14 July 2025

## CITATION

Qiu R, Li J, Jiang C, Yu Y, Li D, Xie X, Lei Y and  
Yao L (2025) Antibacterial activity of baicalein  
against *Aeromonas hydrophila*: *in vitro* and *in*  
*vivo* evaluation. *Front. Microbiol.* 16:1615029.  
doi: 10.3389/fmicb.2025.1615029

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# Antibacterial activity of baicalein against *Aeromonas hydrophila*: *in vitro* and *in vivo* evaluation

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**Introduction:** *Aeromonas hydrophila* (AH) is a pathogenic bacterium commonly found in aquatic organisms, particularly in fish products. Baicalein, a bioactive flavonoid derived from traditional Chinese herbal medicine, possesses a wide range of pharmacological properties, including anticancer, antibacterial, antiviral, antioxidant, hepatoprotective, and anti-inflammatory effects.

**Methods:** *In vitro*, the Oxford cup method was employed to assess the antibacterial activity of baicalein, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the microtiter broth dilution technique. Biofilm formation and bacterial motility were evaluated through the crystal violet assay and swimming assay, respectively. The impact of baicalein on bacterial membrane permeability and morphology was observed using the Live/Dead BacLight kit and transmission electron microscopy. *In vivo*, grass carp were used as the model organism to evaluate the effect of baicalein on AH proliferation, while the expression of pro-inflammatory cytokines and antioxidant factors was quantified by qRT-PCR.

**Results:** This study demonstrated the antibacterial activity of baicalein against AH infection. Baicalein exhibited bacteriostatic effects *in vitro*, with an MIC of 40 µg/mL and an MBC of 80 µg/mL. Time-kill assays confirmed its bactericidal properties. Additionally, baicalein inhibited biofilm formation and reduced bacterial motility. The antibacterial mechanism of baicalein involved increased membrane permeability and structural disruption of AH cells. *In vivo* studies in grass carp revealed a dose-dependent reduction in AH burden following baicalein administration. Moreover, baicalein suppressed the expression of pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α), while enhancing the expression of antioxidant-related genes, such as catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD). These findings indicate that the antibacterial and anti-inflammatory effects of baicalein contribute to its protective role against AH infection *in vivo*.

**Discussion:** Baicalein effectively inhibits the proliferation of AH both *in vitro* and *in vivo*, highlighting its potential as a promising pharmacotherapeutic agent for the prevention of AH infections in fish.

## KEYWORDS

baicalein, *Aeromonas hydrophila*, antibacterial, biofilm, grass carp

## Introduction

*Aeromonas hydrophila* (AH) is a Gram-negative bacterium belonging to the Aeromonadaceae family, commonly found in natural water bodies and recognized as a primary pathogen of various aquatic animals. AH infections have significantly hindered the growth of China's aquaculture industry (Semwal et al., 2023). Traditional strategies for disease prevention and treatment in aquaculture primarily rely on medications and vaccinations. However, the high cost, limited efficacy, and narrow applicability of aquatic vaccines, combined with the rapid mutation of pathogens, have restricted their widespread use. Consequently, drug therapy remains the predominant approach for disease control in aquaculture. Antibiotics are widely used as the primary treatment in aquaculture. However, overuse has contributed to the emergence of antibiotic-resistant bacteria and the horizontal transfer of resistance genes to humans. Additionally, accumulation of antibiotic residuals in aquatic products has also posed significant threats to public health and the environment (Zheng et al., 2021; Zainab et al., 2020). In response, many countries have introduced regulations aimed at reducing or eliminating antibiotic resistance in aquaculture, emphasizing the need for alternative, non-resistant antimicrobial agents (Lulijwa et al., 2020).

Herbal remedies, rich in natural antibacterial compounds, offer promising alternatives to conventional antibiotics due to their efficacy and safety (Vaou et al., 2021; Woo et al., 2023). Various medicinal herbs used in aquaculture have demonstrated antibacterial properties (Zhang et al., 2022). These herbs contain bioactive compounds such as polysaccharides, essential oils, alkaloids, saponins, polyphenols, flavonoids, anthraquinones, and terpenoids, which effectively prevent and treat bacterial infections caused by *Aeromonas* spp., *Streptococcus* spp., *Vibrio* spp., and other pathogens (Zhu, 2020).

Baicalein, a flavonoid (Figure 1), is a major active component of *Scutellaria baicalensis* (commonly known as Baikal skullcap) (Semwal et al., 2023). It has been well known for its low toxicity and diverse pharmacological effects, including anticancer, antibacterial, antiviral, hepatoprotective, anti-inflammatory, hypoglycemic, antithrombotic, neurogenic, cardioprotective, and wound-healing properties (Paul et al., 2024). Baicalein has demonstrated broad-spectrum antibacterial activity against

both Gram-positive and Gram-negative bacteria, including ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Morimoto et al., 2023), as well as cariogenic, periodontopathogenic bacteria (Jang et al., 2014), and *Helicobacter pylori* (Chen et al., 2018). As for the aquaculture disease aspects, the extract from aerial part of *Scutellaria baicalensis* can inhibit the growth of several common pathogenic bacteria in aquaculture that including *Aeromonas hydrophila*, *Edwardsiella tarda*, *Vibrio alginolyticus* and *Vibrio harveyi* (Xia et al., 2020). It can also serve as a replacement for antibiotics in fighting against pathogenic bacteria (Xia et al., 2023). Baicalein can improve growth performance, antioxidant activity, and intestinal flora of koi carp (*Cyprinus carpio*) (Du et al., 2022). However, no prior study has evaluated baicalein's efficacy against AH.

In this study, we investigated the antibacterial effects of baicalein on AH, focusing on its ability to inhibit bacterial growth and biofilm formation *in vitro*. Additionally, we explored its *in vivo* antibacterial, anti-inflammatory, and antioxidant effects in grass carp (*Ctenopharyngodon idella*). The findings suggest that baicalein holds potential as a plant-derived therapeutic agent for controlling AH infections in aquaculture species.

## Materials and methods

### Fish and bacterial preparation

Grass carp (*Ctenopharyngodon idella*) (81.6 g  $\pm$  8.9 g) were obtained from a local fish farm in Nanyang, Henan, China. The fish were acclimated in aerated freshwater at 23  $\pm$  1°C for 2 weeks prior to experimentation. To confirm the absence of bacterial infections, blood, liver, and kidney samples were examined before the experiments according to previous report (Qiu et al., 2021). The fish were euthanized using 200 mg/L MS-222 (Sigma, St. Louis, MO, USA) before the sacrifice or infection experiments (Qiu et al., 2021) and approved by the institutional Animal Care and Use Committee (Protocol NYNU-2022-016) to ensure animal welfare. The bacterial pathogen *A. hydrophila* (AH) was isolated from grass carp and cultivated at Nanyang Normal University, China. AH was cultured in Luria-Bertani (LB) broth at 28°C.

### Antibacterial activity

The antibacterial activity of baicalein (Aladdin, Shanghai, China) against AH was evaluated using the Oxford cup diffusion method. Baicalein was dissolved in dimethyl sulfoxide (DMSO) (Kemiu, Tianjin, China) at concentrations of 5 mg/mL and 10 mg/mL. 10 mg/mL kanamycin was used as a positive control. AH was adjusted to 1  $\times$  10<sup>7</sup> colony-forming units (CFU)/mL in phosphate-buffered saline (PBS) and spread onto LB agar plates. Sterile Oxford cups were used to create wells in the agar, into which 50  $\mu$ L of baicalein solution was added. The plates were incubated at 28°C for 16–20 h, and antibacterial activity was assessed by measuring the diameter of the inhibition zones.

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. Baicalein concentrations

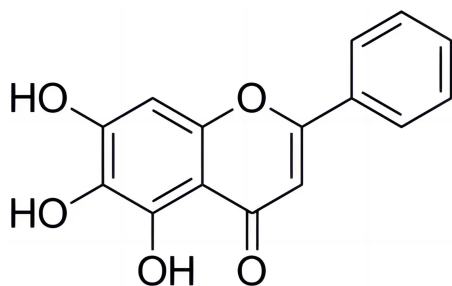


FIGURE 1  
Chemical structure of baicalein. The IUPAC name of baicalein is 5,6,7-Trihydroxy-2-phenylchromen-4-one, or also known as 5,6,7-trihydroxyflavone.

ranging from 320 µg/mL to 1.25 µg/mL were prepared with 198 µL of AH suspension and 2 µL of the twofold serial dilutions of baicalein in 96-well plates. Wells were inoculated with AH at  $1 \times 10^6$  CFU/mL and incubated at 28°C for 24 h. The control group was set up by adding the same volume of DMSO and LB medium. Three parallel experiments were conducted for each group. MIC was defined as the lowest concentration at which no turbidity was observed. The minimum bactericidal concentration (MBC) was determined by plating 100 µL from clear wells onto LB agar and incubating at 28°C for 24 h. MBC was defined as the lowest baicalein concentration that killed 99.9% of the bacterial population, following CLSI M07-A8 guidelines. All assays were conducted in triplicate in two independent experiments.

## Time-kill assay

Time-kill kinetics of baicalein against AH were evaluated by culturing AH in LB broth at  $1 \times 10^6$  CFU/mL and treating it with baicalein at concentrations of 10, 20, 40, and 80 µg/mL. Cultures were incubated at 28°C, and bacterial viability was assessed at 0, 2, 4, 6, 8, 12, and 24 h post-treatment by determining CFU/mL. Experiments were conducted in triplicate.

## Biofilm formation assay

The effect of baicalein on biofilm formation was assessed using a crystal violet assay (Solarbio, Beijing, China) in a 96-well plate as previously described (Bhattacharyya et al., 2017). AH ( $2 \times 10^5$  CFU/mL) was incubated in LB broth with baicalein at 2.5, 5, 10, 20, and 40 µg/mL for 72 h at 28°C. Biofilms were fixed with 100% methanol for 15 min, air-dried, and stained with 0.1% crystal violet for 20 min. After washing, the bound dye was solubilized with 30% glacial acetic acid, and biofilm formation was quantified by measuring optical density at 570 nm (OD<sub>570</sub>). Biofilm formation was normalized using OD<sub>600</sub> (bacterial concentration), calculated as: biofilm OD<sub>570</sub> (relative value) = OD<sub>570</sub> (measured value)/OD<sub>600</sub> (bacterial concentration).

## Motility assay

Bacterial motility was assessed using a modified method (Husain et al., 2017). Soft LB agar (0.3%) was autoclaved at 121°C for 30 min. After cooling to 55°C, baicalein was added to achieve final concentrations of 2.5, 5, 10, 20, and 40 µg/mL before plating. AH cultures were washed with PBS, adjusted to  $1 \times 10^7$  CFU/mL, and inoculated (2 µL) onto the agar surface. Plates were incubated at 28°C for 24 h, and colony diameters were measured. Each assay was performed in triplicate.

## Membrane permeability assay

A Live/Dead BacLight kit (Solarbio, Beijing, China) was used to assess membrane permeability. Log-phase AH cultures were treated

with baicalein ( $2 \times$  MIC) for 2 h, washed with 0.85% NaCl, and centrifuged at  $3,000 \times g$  for 5 min. Control samples were incubated without baicalein. Bacterial suspensions ( $1 \times 10^8$  CFU/mL) were stained with SYTO 9 and propidium iodide (PI) and incubated in the dark for 15 min. Fluorescence images were captured using a Zeiss Axio Observer 7 microscope (Zeiss, Jena, Germany).

## Transmission electron microscopy

The structural effects of baicalein on AH were investigated by a Hitachi HT7700 Transmission Electron Microscope (Hitachi, Tokyo, Japan). Briefly, AH cultures were treated with baicalein ( $2 \times$  MIC) for 6 h at 28°C, washed with PBS, and centrifuged at  $3,000 \times g$  for 5 min. Cells were fixed in 2.5% glutaraldehyde at 4°C overnight, washed, and air-dried before TEM analysis. DMSO-treated bacteria served as controls.

## Bacterial burden assay

Grass carp were intraperitoneally injected with 0.1 mL of AH ( $1 \times 10^6$  CFU/mL). After 2 h, fish were randomly assigned to five groups ( $n = 5$ ) and administered baicalein (10 mg/kg, 5 mg/kg, or 2.5 mg/kg), DMSO, or PBS via intraperitoneal injection. Fish were euthanized after 4 h, and liver and spleen samples were collected, weighed, and homogenized in PBS. Serial dilutions were plated on LB agar and incubated at 28°C for 24 h. Bacterial counts were expressed as CFU/g of tissue.

## RNA extraction and qRT-PCR

The expression of inflammation-related genes (Table 1), including interleukin-1β (*IL-1β*), interleukin-8 (*IL-8*), and tumor necrosis factor-α (*TNF-α*) and antioxidant-related genes catalase (*CAT*), glutathione reductases (*GR*) and superoxide dismutase (*SOD*) in liver tissue was analyzed via qRT-PCR. Total RNA was extracted using the Trizol method and reverse-transcribed into cDNA with Superscript II reverse transcriptase (Takara, Dalian, China). qRT-PCR was performed using a SYBR Green Pro Taq qPCR Kit (Agbio, Changsha, China) with *β-actin* as the internal control. The PCR reaction system comprised 10 µL, with 5 µL of SYBR, 0.5 µL of each primer, 3.5 µL of DEPC water, and 1 µL of cDNA as the template. The PCR conditions were: 95°C for 5 min, and followed by 40 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 15 s. The relative gene expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method.

## Statistical analysis

Data were analyzed using one-way ANOVA in SPSS 27.0. Statistical significance was set at  $P < 0.05$  (\* or #) and  $P < 0.01$  (\*\* or ##). Graphs were generated using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA).

TABLE 1 Primers used in this study.

| Primer name        | Primer sequence (5'-3')  | GenBank accession |
|--------------------|--------------------------|-------------------|
| qIL-1 $\beta$ -F   | CTGGTCTTGGAGGAGGTCACTGAA | JN705663.2        |
| qIL-1 $\beta$ -R   | CTACTTGGCACCTGGCACACTTC  |                   |
| qIL-8-F            | GCTGTGGCATGTCTGACCATTACT | JN255694.1        |
| qIL-8-R            | ACAGTGAGGGCTAGGAGGGTAGA  |                   |
| qTNF- $\alpha$ -F  | TGCTGTCTGCTTCACGCTCAAC   | JQ670915.1        |
| qTNF- $\alpha$ -R  | AGCCTGGTCTGGTTCACTCTC    |                   |
| qCAT-F             | TGCCTGTCAACTGCCCTAC      | FJ560431.2        |
| qCAT-R             | CGGCTTCGTTTCAGCACCTC     |                   |
| qGR-F              | CTTTGAACCTGAATCTTGCCCTAA | JX854448.1        |
| qGR-R              | CCCAGAGCAGACAGTCCACC     |                   |
| qSOD-F             | TGTGGACAAAATGCTGACCCTG   | GU901214.1        |
| qSOD-R             | TTCCTCATTCGCTCCCTTCC     |                   |
| q $\beta$ -actin-F | TTGCCGCACTGGTTGTTG       | M25013.1          |
| q $\beta$ -actin-R | TCCGTTTCTCACCTGATGTCTG   |                   |

Results

Baicalein’s anti-AH efficacy *in vitro*

Baicalein exhibited significant antibacterial activity against *A. hydrophila* (AH). At concentrations of 5 mg/mL and 10 mg/mL, the inhibition zones measured  $18.47 \pm 0.40$  mm and  $20.08 \pm 0.18$  mm, respectively, compared to  $23.26 \pm 0.39$  mm for 10 mg/mL kanamycin (positive control; Figure 2). The MIC and MBC values were determined to be 40  $\mu$ g/mL and 80  $\mu$ g/mL, respectively. Notably, at concentrations  $\geq 80$   $\mu$ g/mL, no bacterial colonies were observed, demonstrating a potent bactericidal effect.

Time-kill analysis

Baicalein inhibited AH growth in a dose- and time-dependent manner. The time-kill curves (Figure 3) showed a substantial decrease in viable bacterial counts with increasing baicalein concentration. At 80  $\mu$ g/mL (MBC), AH was completely eradicated within 12 h. At 40  $\mu$ g/mL (1 $\times$  MIC), a significant reduction in bacterial counts was observed within the first 12 h, though some bacterial cells survived and proliferated by 24 h. These findings suggest that baicalein’s antibacterial efficacy is dependent on both concentration and exposure time.

Effects of baicalein on biofilm formation and motility

The crystal violet assay demonstrated that baicalein significantly reduced biofilm formation in a concentration-dependent manner, as indicated by a lower OD<sub>570</sub> (relative value) in baicalein-treated groups compared to the DMSO-treated group (Figure 4). Biofilm inhibition ranged from 20.9% to 32.7% at

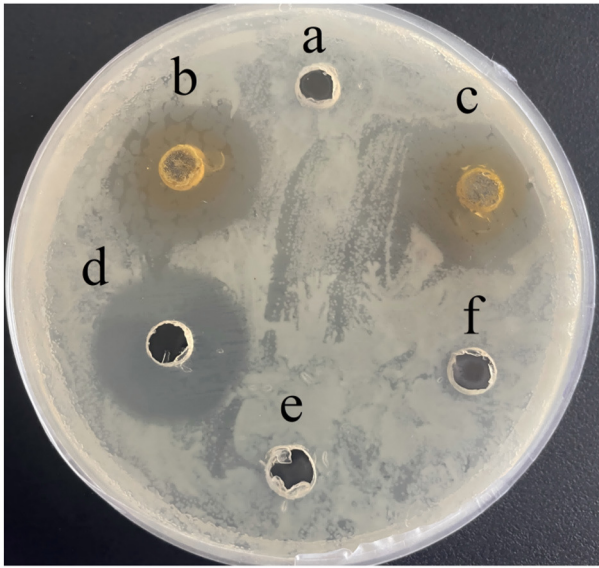


FIGURE 2  
Inhibition zone of baicalein against AH *in vitro*. (a), DMSO; (b), 5 mg/mL baicalein; (c), 10 mg/mL baicalein; (d), 10 mg/mL kanamycin; (e), PBS; (f), H<sub>2</sub>O.

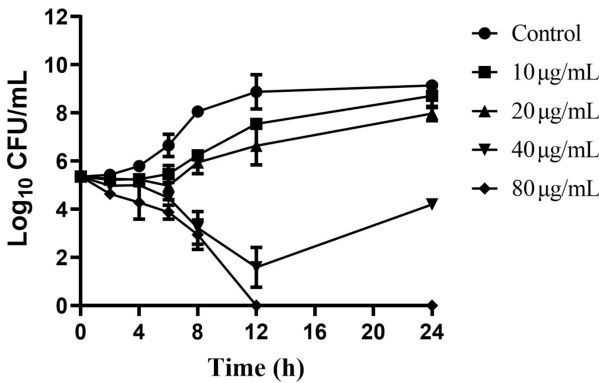
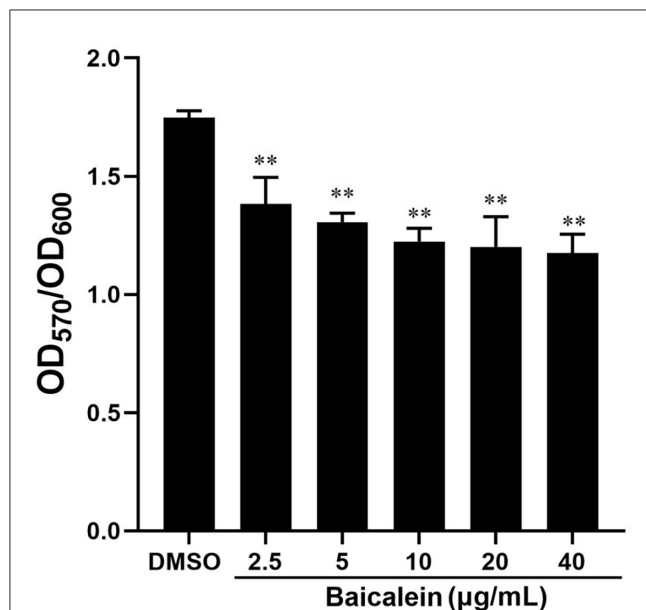


FIGURE 3  
Time-kill curves of AH vs. different concentrations of baicalein. The baicalein concentrations were 10, 20, 40, and 80  $\mu$ g/mL (as 1/4, 1/2, 1, and 2 MIC). Samples were taken at 0, 2, 4, 6, 8, 12, and 24 h to determine viable bacterial numbers.

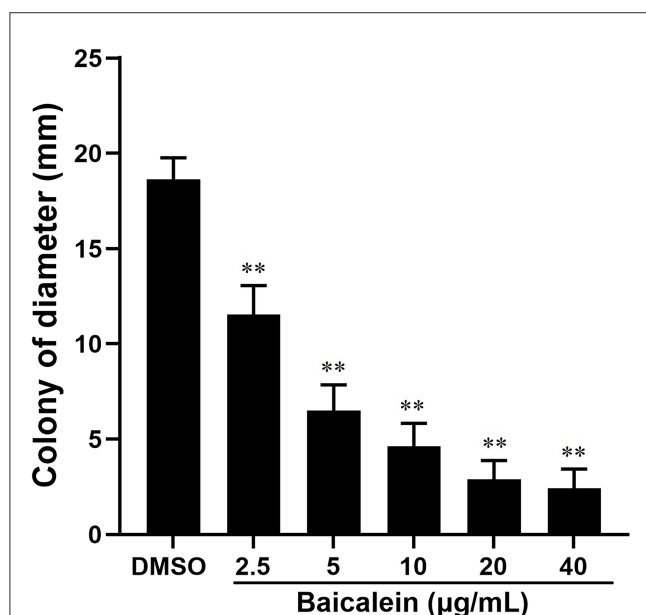
concentrations between 2.5 and 40  $\mu$ g/mL. Additionally, baicalein significantly impaired AH motility on semi-solid agar, as evidenced by reduced colony diameters in treated groups compared to controls (Figure 5).

Baicalein disrupts the membrane structure of AH

Live/Dead BacLight bacterial viability assays indicated that baicalein increased membrane permeability, as evidenced by enhanced red fluorescence in treated AH cells (Figure 6). Transmission electron microscopy (TEM) analysis further revealed



**FIGURE 4**  
Effect of baicalein on the formation of AH biofilms. Different concentrations of baicalein (2.5, 5, 10, 20, and 40 µg/mL) were incubated with AH for 72 h. The biofilm formation was tested by the crystal violet staining method. \*\* $P < 0.01$  compared to the DMSO treated group.



**FIGURE 5**  
Effect of baicalein on the motility of AH. The assays were conducted on plates containing 0.3% agar in the presence of different concentrations of baicalein (2.5, 5, 10, 20, and 40 µg/mL) or DMSO as a control. The AH was incubated for 24 h. Colony diameters were measured using a caliper. \*\* $P < 0.01$  compared to the DMSO treated group.

that baicalein treatment caused significant structural damage, including membrane distortion and cell wall folding (Figure 7), whereas control cells maintained their structural integrity. These

findings suggest that baicalein may inhibit AH growth by compromising membrane integrity and inducing cellular damage.

## In vivo antibacterial efficacy of baicalein

The antibacterial activity of baicalein was evaluated using an AH-infected grass carp model. Baicalein treatment significantly reduced bacterial counts in both liver and spleen tissues in a dose-dependent manner. In the liver, baicalein at 2.5, 5, and 10 mg/kg resulted in bacterial counts that were 3.9-, 2.3-, and 1.1 orders of magnitude lower than those in the DMSO-treated group, respectively (Figure 8a). In the spleen, bacterial counts were reduced by 6.5-, 6-, and 1.2 orders of magnitude lower at the corresponding baicalein doses (Figure 8b). These results demonstrate the potent *in vivo* bactericidal effect of baicalein against AH.

## Anti-inflammatory effects of baicalein in liver tissues

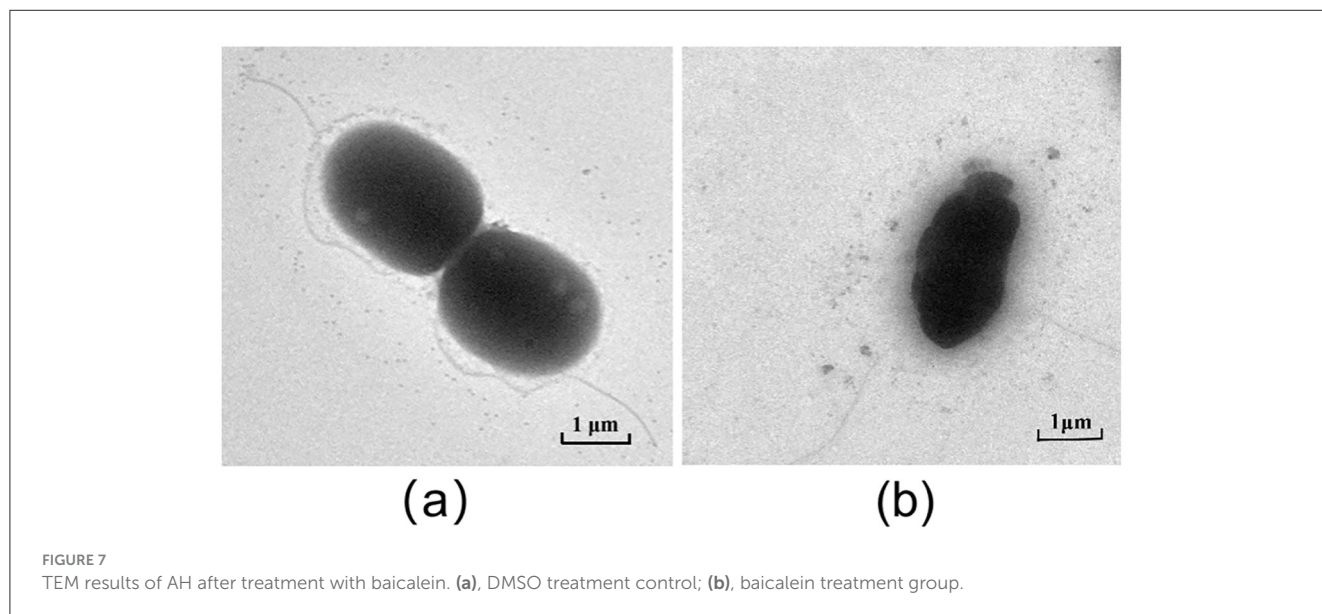
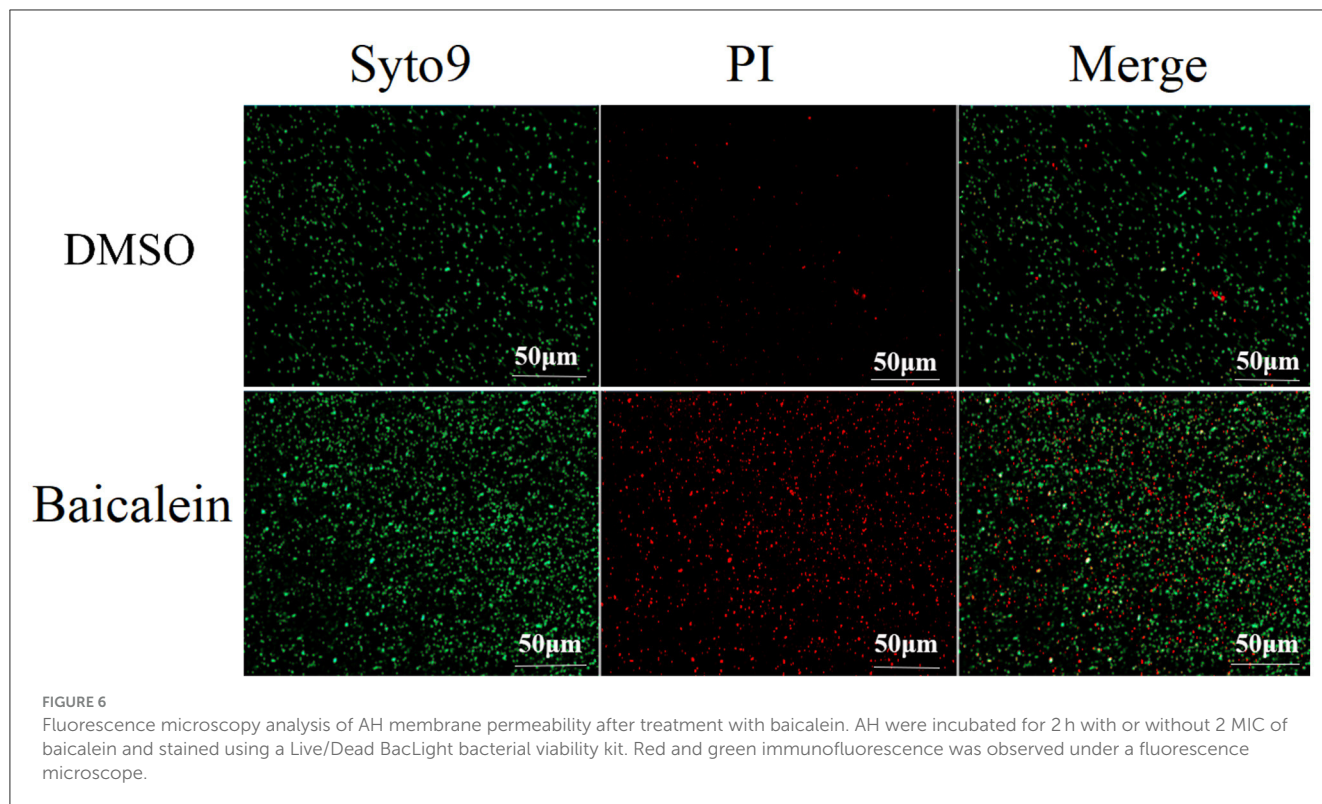
qRT-PCR analysis revealed that baicalein treatment led to a dose-dependent suppression of inflammatory cytokine expression (*IL-1β*, *IL-8*, and *TNF-α*) in liver tissues following AH infection. Compared to the AH-infected control group, *IL-1β* expression levels were 1.5-, 7.8-, and 6.8-fold lower in fish treated with 2.5, 5, and 10 mg/kg baicalein, respectively (Figure 9a). Similarly, *IL-8* expression levels were reduced by 0.88-, 7.9-, and 8.2-fold (Figure 9b), and *TNF-α* expression was decreased by 0.95-, 2.6-, and 4.2-fold, respectively (Figure 9c). These results suggest that baicalein effectively modulates AH-induced inflammatory responses in the liver.

## Upregulation of antioxidant-related genes by baicalein

qRT-PCR analysis showed a significant increase in the mRNA expression of antioxidant-related genes (*CAT*, *GR*, and *SOD*) in baicalein-treated liver tissues compared to the AH-infected group. Specifically, *CAT* expression increased by 1.1-, 14.1-, and 29.1-fold in fish treated with 2.5, 5, and 10 mg/kg baicalein, respectively (Figure 10a). *GR* expression was elevated by 3.4-, 3.8-, and 8.1-fold (Figure 10b), and *SOD* expression increased by 6.4-, 8.4-, and 13.1-fold (Figure 10c). These findings indicate that baicalein enhances antioxidant responses in the liver, suggesting its potential role in oxidative stress regulation.

## Discussion

Baicalein, a major bioactive compound derived from *Scutellaria baicalensis*, has been widely studied for its pharmacological effects, particularly in infectious disease control. In our current study, baicalein exhibited significant antibacterial effects against AH, with MIC and MBC values of 40 µg/mL and 80 µg/mL, respectively.



Previous studies reported MIC values of baicalein against *S. aureus* (64–256 µg/mL) (Chan et al., 2011; Chen et al., 2016) and *P. aeruginosa* (>1,024 µg/mL) (Luo et al., 2016). As some Chinese Herbal Medicine, the MIC and MBC of thymol to AH were 128 µg/mL and 256 µg/mL, respectively (Liang et al., 2022). The MIC of the water extract from gall, coptis, terminalia, rhubarb to AH were 7.81 mg/mL, 15.63 mg/mL, 31.25 mg/mL, 62.50 mg/mL, respectively. The MBC were 15.63 mg/mL, 62.50 mg/mL, 62.50 mg/mL, 125.00 mg/mL, respectively (Su et al., 2012). The MIC values of antibiotics emodin and enrofloxacin against AH were

found to be 100 µg/mL and 9.375 µg/mL respectively (Zhang et al., 2014). Baicalein demonstrated superior antibacterial activity against AH than other Chinese Herbal Medicine and closed to the antibiotics. The time-kill assay further confirmed its bactericidal properties at 80 µg/mL. Biofilm formation is a major contributor to antibiotic resistance (Rather et al., 2021). Using the crystal violet assay, our findings revealed that baicalein significantly inhibited AH biofilm formation and bacterial motility in a concentration-dependent manner, similar to previous reports on *S. aureus* (Chen et al., 2016) and *Streptococcus mutans* (Vijayakumar et al.,

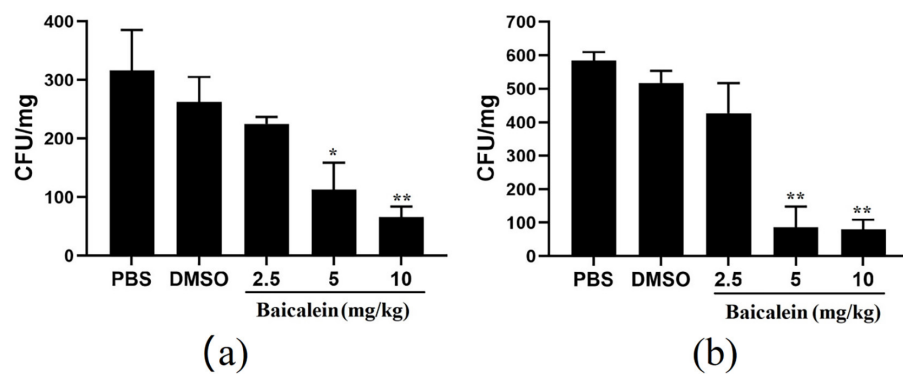


FIGURE 8

The number of colonies in the liver (a) and spleen (b) of grass carp after challenged with AH. \* $P < 0.05$  and \*\* $P < 0.01$  indicate significantly different from the DMSO group.

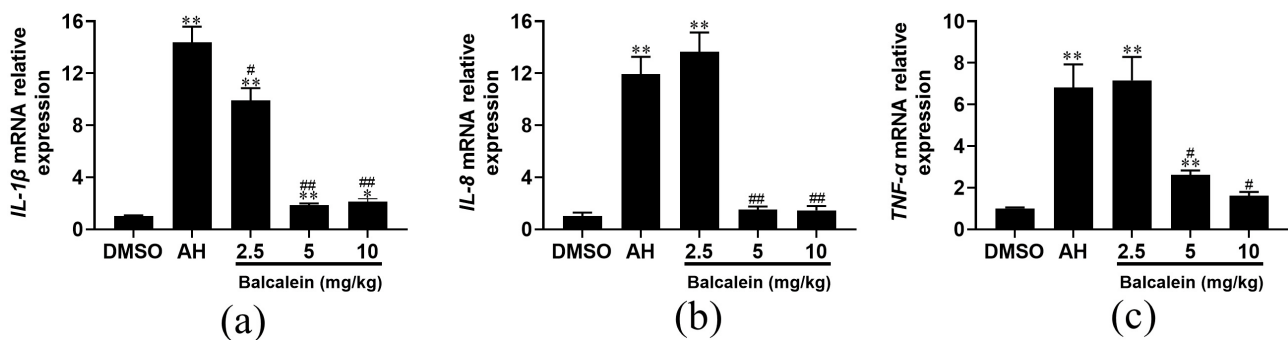
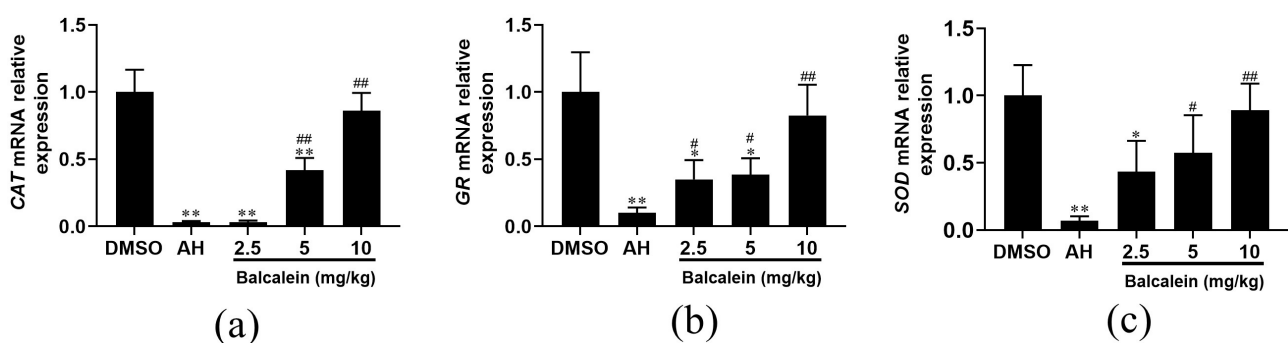


FIGURE 9

The mRNA expression levels of inflammatory genes after treatment with baicalein. The mRNA expression levels of *IL-1β* (a), *IL-8* (b), and *TNF-α* (c) were detected by qRT-PCR. Expression values are normalized against  $\beta$ -actin expression and data are expressed as the mean fold change from the control group or AH group. Three biological replicates were performed and the data are presented as mean  $\pm$  SD ( $n = 3$ ). \* $P < 0.05$ , \*\* $P < 0.01$  indicate significantly different from the DMSO-treated group; # $P < 0.05$  and ## $P < 0.01$  indicate significantly different from the AH group.



the inhibition of AH motility by baicalein suggests a potential mechanism for biofilm disruption. Furthermore, baicalein was found to increase membrane permeability and disrupt AH cell membranes, consistent with prior studies on *S. aureus* (Wang et al., 2023; Paul et al., 2024). Many studies show that baicalein is a safe, low toxicity and few adverse effects (Paul et al., 2024). Studies have shown that the safe dosage is 500mg/kg in rat (Li et al., 2014). We use the dose of 10 mg/kg baicalein in the grass carp. The fish remains healthy with no adverse symptoms. *In vivo*, baicalein significantly reduced AH burden in grass carp, aligning with its reported efficacy against *H. pylori* (Chen et al., 2018) and *S. suis* infections in mice (Lu et al., 2021). Therefore, our findings indicated that baicalein could potentially be used as an antibiotic adjuvant drug for preventing aquatic animal infectious diseases caused by antibiotic-resistant bacteria (Güran et al., 2023; Liu et al., 2020; Wang et al., 2023). However, baicalein uses in aquaculture is limited by its poor water solubility, low bioavailability, and rapid breakdown in fish. It also degrades in alkaline water and is poorly absorbed when given orally. Direct water application is ineffective due to quick dilution. To improve its effectiveness, advanced delivery methods like nanoencapsulation are being explored to enhance stability, absorption, and targeting (Wang et al., 2024).

Microbial infections are regulated by host inflammatory responses, primarily driven by innate immunity. In aquaculture, *A. hydrophila* (AH)-induced sepsis poses a significant threat, as it triggers a systemic inflammatory response that can lead to severe tissue damage (Semwal et al., 2023). Pro-inflammatory cytokines play a crucial role in immune signaling cascades, with *IL-1 $\beta$* , *IL-8*, and *TNF- $\alpha$*  being key mediators of inflammation (Megha et al., 2021). The liver is a central organ involved in immune regulation and systemic inflammation in fish (Wu et al., 2016). Moreover, previous studies have shown that baicalein exerts liver-protective effects (Yang et al., 2021), making it a relevant tissue for evaluating its anti-inflammatory potential. In this study, we analyzed inflammation levels in fish and found that AH infection significantly upregulated the expression of *IL-1 $\beta$* , *IL-8*, and *TNF- $\alpha$* . However, baicalein treatment markedly suppressed the AH-induced release of these cytokines in the liver. Previous research has demonstrated that baicalein reduces bacterial burden by inhibiting the expression of *IL-1 $\beta$* , *IL-8*, *TNF- $\alpha$* , and other inflammatory mediators, thereby mitigating excessive inflammatory responses in severe abdominal sepsis (Chen et al., 2022; Guo et al., 2019; Wang et al., 2023). These findings suggest that baicalein may exert a protective effect against AH-induced damage by attenuating inflammation.

Aquatic organisms produce various antioxidant enzymes and compounds in response to environmental stressors to counteract oxidative damage caused by reactive oxygen species (ROS) and other oxidants (Yang et al., 2022). Maintaining redox homeostasis is a critical component of the defense mechanisms that organisms have evolved to mitigate ROS-induced oxidative damage (Jiang et al., 2024). CAT and SOD are enzymatic antioxidants that play essential roles in neutralizing ROS (Rattanawong et al., 2021). Additionally, GR is involved in the glutathione redox cycle, facilitating the elimination of free radicals and peroxides while enhancing cellular resistance to oxidative stress (Chowdhury et al., 2020). Previous studies have shown that AH infection

can induce excessive ROS production (Han et al., 2020), while baicalein exhibits antioxidative effects through its ROS-scavenging properties (Pan et al., 2021). In this study, we found that grass carp challenged with AH exhibited significantly reduced expression levels of SOD, CAT, and GR in liver tissues compared to the control group. However, baicalein treatment significantly upregulated the expression of these antioxidant-related genes, indicating its potential role in enhancing the antioxidant defense system in response to AH-induced oxidative stress.

It is important to note that while our study demonstrates transcriptional changes in inflammation- and antioxidant-related genes, it does not directly assess oxidative damage through biochemical markers such as malondialdehyde (MDA) (Rizzo, 2024). Future studies should incorporate such assays to more comprehensively evaluate the antioxidant efficacy of baicalein *in vivo*.

## Conclusions

Baicalein demonstrated potent antibacterial activity against AH *in vitro*, inhibiting bacterial motility, preventing biofilm formation, and increasing membrane permeability, ultimately leading to bacterial cell death due to cell structural damage. *In vivo*, baicalein effectively reduced AH burden, suppressed inflammatory responses, and enhanced antioxidant capacity in grass carp. These findings highlight baicalein's potential as a natural antimicrobial agent for controlling AH infections in aquaculture species.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository and accession numbers can be found in the Table 1.

## Ethics statement

The animal study was approved by the Animal Experiment Ethics Committee of Nanyang Normal University (Approval No. NYNU-2022-016). The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

RQ: Investigation, Conceptualization, Resources, Writing – review & editing, Funding acquisition, Project administration, Writing – original draft, Methodology, Visualization, Formal analysis, Software. JL: Writing – review & editing, Methodology, Visualization. CJ: Methodology, Writing – review & editing, Data curation, Validation. YY: Writing – review & editing, Validation. DL: Writing – review & editing, Data curation. XX: Formal analysis, Writing – review & editing. YL: Validation, Methodology, Data curation, Writing – review & editing. LY: Funding acquisition, Writing – review & editing, Conceptualization, Supervision.

## Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was partially supported by the Henan Province Central Leading Local Science and Technology Development Fund (Z20221343035), the Doctoral Research Project of Nanyang Normal University (2023QN009), and the Research and Practice Project on Research based Teaching Reform in Undergraduate Universities in Henan Province (2022SYJXLX079).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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