Experimental Study on the Inhibition of Bacteria and Algae by *Jussiaea stipulacea* Ohwi Extract

Yulu Shi¹, Yao Zheng¹,², Xuwen Bing¹,² and Julin Yuan³

Abstract

Nowadays, plant allelopathy, as a new type of biological algal and/or bacterial inhibition technology, has attracted extensive attention. Eight substances were isolated and identified from *Jussiaea stipulacea* Ohwi, and five concentration gradients, as well as a control (0, 1.25, 5, 10, 20, and 50 mg/L) were set, with three parallels in each group, and then sampled and detected at 24, 48, 72, and 96 h. When the concentration was 50 mg/L, the inhibition rate of *Anabaena* was as high as 74.8%, 69.2%, and 70.7% for ursolic acid, kaempferol, and luteolin, respectively. 

**Keywords**

allelopathic substances, *Jussiaea stipulacea* Ohwi, erythromycin, florfenicol, bacteriostatic and algal control

Received: May 4th, 2022; Accepted: August 22nd, 2022.

Introduction

At present, water pollution caused by algal blooms due to eutrophication has become an issue of global concern. *Aeromonas hydrophila* and *Streptococcus iniae* can cause serious health problems for fish, increase the mortality for aquaculture, and decrease the quality of food. In order to prevent and control algal growth and bacterial diseases, antibiotics such as erythromycin and florfenicol are widely used in aquaculture.¹ However, the wide use of antibiotics will make pathogens resistant, and it is generally recommended to use aquatic plant extracts to treat water pollution and bacterial diseases during aquaculture. Herbs with potential inhibitory effects on algae and bacteria play a key role in aquaculture disease management.² It is reported that herbal extracts such as *Acorus calamus*, Cattail, and *Zanthoxylum bungeanum* can effectively inhibit pathogenic bacteria.³ The bacteriostatic and algicidal effects of allelochemicals secreted by aquatic plants have attracted much attention worldwide.⁴ In addition, plants can absorb nutrients in the water and provide channels for the export of nutrients from the water. Conversely, they can secrete secondary metabolites, like allelochemicals, that inhibit algal growth and play a role in purifying eutrophic water. They can be degraded under natural conditions, do not accumulate for a long time in the ecosystem, and are relatively ecologically safe.⁵ Another study reviewed the mechanism of plant allelopathic inhibition of algal growth.⁶ A different study focused on the potential applications in algal inhibition by aquatic plant allelopathy.⁷

Our previous study selected several Chinese medical plants for nutrient removal via floating bed cultivation in aquatic ponds⁸,⁹ (ie *Jussiaea stipulacea* Ohwi). Based on the fact that this plant is yellow in water due to the accumulation of allelochemicals, Wu et al found that these had a strong inhibitory effect on *Microcystis aeruginosa* (FACHB-905). Within 7 days of culture, the optimum concentration range for algal inhibition...
was 50-75 mg/L, and the relative inhibition rate of algal cells was 50%-95% within 4-7 days of culture. The allelochemicals were isolated to explore their inhibitory effect on the growth of *M. aeruginosa, Euglena*, and *Anabaena*, the range of their optimal inhibitory concentration, as well as their inhibitory effect and minimum inhibitory concentration on *A. hydrophila* and *S. iniae*. The values were compared with the bacteriostatic and algicidal effects of erthyromycin and florfenicol. The results of this study can be used as a reference for disease control and health management of aquaculture.

Materials and Methods

**Materials, Extraction, and Identification**

The pathogenic bacteria *S. iniae* and *A. hydrophila* (WJ-8) were identified and prepared by our laboratory from FFRC-CAFS. *M. aeruginosa, Euglena*, and *Anabaena* were purchased from the freshwater algae species bank at the Chinese Academy of Sciences (CAS), China. Dry *J. stipulacea* (2 kg) plants were extracted three times with eight times the amount of water, with each extraction taking approximately 4 days. The extracts were concentrated under reduced pressure to give a total dry extract of 210 g. This was dissolved in water, and then extracted with ethyl acetate and *n*-butanol (Sigma-Aldrich, St Louis, MO, USA). After recovery of the solvent, 103 g of ethyl acetate and 65 g of *n*-butanol dry extracts were obtained. The ethyl acetate extract (20 g) was separated by silica gel column chromatography by elution with a light petroleum-ethyl acetate gradient. The 20:1 fraction was rechromatographed on a silica gel column using gradients (0, 1, 5, 10, 20:1) to produce compound II (15 mg, Figure S1b). The 8:1 fraction yielded a crude powder, which, after recrystallization from light petroleum-ethyl acetate, gave colorless acicular crystals of II (150 mg, Figure S1b). The ethyl acetate extract (20 g) was separated by silica gel column chromatography using a light petroleum-ethyl acetate gradient. The 50:1 fraction was further separated by silica gel column chromatography, eluting with light petroleum-ethyl acetate (20:1) to give compound III (8 mg, Figure S1c). The isolated fraction via chloroform-methanol (10:1) was separated by silica gel column chromatography, and a white powder was eluted with a chloroform-methanol gradient. Then the white acicular crystal IV (20 mg, Figure S1d) was obtained by Sephadex LH-20 column chromatography and isolated from the methanol elution. Compound V (8 mg, Figure S1e) was obtained by further separation of the chloroform-methanol 8:1 eluate by preparative HPLC. The chloroform-methanol 10:1-5:1 eluate was further purified by preparative HPLC to obtain compound VI, as a yellow powder (20 mg, Figure S1f). The *n*-butanol extract (20 g) was separated by silica gel column chromatography by eluting with a chloroform-methanol gradient. The 10:1 fraction yielded a yellow powder, which was further purified by preparative HPLC to obtain substance VII (16 mg, Figure S1g). The 8:1 fraction was further purified by preparative HPLC to obtain substance VIII (Figure S1h). Compounds III and VII showed no significant antimicrobial and anti-algal effects and were not studied further.

Sliced *J. stipulacea* leaves (50 g) were soaked in 150 g distilled water for 2 days, after which they were placed in a 4 °C refrigerator and stirred three times a day. The mixture was then filtered three times with two layers of gauze, then with filter paper and microporous filter membranes (Sigma-Aldrich) to eliminate the interference of microorganisms. The extract was concentrated to 50 g/L and stored in a refrigerator at 4 °C.

The content of each component in the raw materials and the ethyl acetate extract was determined by HPLC using an Agilent 1200 system (Agilent Technologies, Waldbronn, Germany), equipped with a G1322A online vacuum degasser, a G1311A quaternary pump, a G1329A autosampler, a G1314B UV detector, and Chemstation software (Agilent Technologies).

**Algal Inhibition Activity of J. stipulacea Extract**

After receiving the algal seed, the mixture was shaken well, transferred to an aseptic glass triangle bottle under the operation of an aseptic super cleaning table, sealed with film, and cultured in a light incubator. The culture temperature was 25 °C, the light condition 1000-2000 Lux, the light-to-dark ratio 12 h:12 h, and the triangle bottle was shaken twice a day. The algal species had a substantial growth rate, and the biomass increased obviously, which could then be transferred again at a ratio of 1:5. Microscopes were often used to check the growth of algae, and the culture medium was added irregularly for continuous seed expansion. The BG-11 culture medium for *M. aeruginosa, Anabaena*, and the HUT culture medium for Gymnocalgae were purchased from the freshwater algae species library at the Institute of Hydrobiology, CAS. All experimental procedures were carried out under aseptic conditions. The algal cells cultured in the stable stage were diluted into different multiples, and the algal density was counted under a microscope using a blood cell counting board and plankton counting frame; cell counting was carried out three times. The corresponding optical density of the algal cells was determined by ultraviolet spectrophotometry. A linear relationship was established between the algal density and optical density. The wavelength of the optical density value was scanned using an ultraviolet spectrophotometer, and the position of the wave peak was selected as the optimal wavelength. The prepared *J. stipulacea* extract was added to three different algal solutions, and the blank control was set. The mass concentration of *J. stipulacea* extract was set to five gradients (0, 1, 5, 10, and 25 g/L), and three replicates were set for each group. The effect of the extract on algal growth was tested by measuring the absorbance of the algal mixture. The total volume of the algal mixture was 80 mL. The initial cell concentration of *M. aeruginosa* was 1 × 10^6 cells/mL, and that of *Anabaena* and *Euglena* 1 × 10^3 cells/mL. Samples (1 mL) were taken every 24 h (0, 24, 48, 72, and 96 h) to determine the absorbance of the algal solution and calculate the concentration of algal cells.
Algal Inhibition Activity of Standard Allelochemicals from J stipulacea

The prepared J stipulacea extract was added to the three algal suspensions, respectively, and the drug concentration gradient in these was 0, 1.25, 5, 10, 20, and 50 mg/L, with three parallels in each group. The algal inoculum was placed in a light incubator at 25 ± 1 °C, a light-to-dark ratio of 12 h:12 h, and a light intensity of 1000-2000 Lux. The algal inoculum mixture was shaken twice a day and the position was exchanged to receive light evenly. Next, 1 mL samples were taken at 24, 48, 72, and 96 h, and a UV spectrophotometer (BioTek) was used to measure the absorbance and calculate the concentration of algal cells at wavelengths from 400 to 950 nm to determine the most effective absorption wavelength. A standard curve was produced for quantitative calculation. The formula for algal growth inhibition rate was as follows: \( IR = \frac{(C - T)C \times 100}{C} \), in which “C” represents the density of algal cells in the control group and “T” represents the density of algal cells in the experimental group.

Antibacterial Activity of Standard Allelochemicals from J stipulacea

After gradient dilution, 100 μL of the diluted bacterial solution was coated onto a solid medium. After 24 h, the colonies were counted to determine the concentration of the bacterial solution. S iniae and A hydrophila (isolated from a fish farm and stored in our lab) were cultured to a logarithmic phase and diluted to \( 1 \times 10^8 \) CFU/mL bacterial suspension in a liquid medium. Bacterial suspension (150 μL) and 15 μL of the different concentrations of the drug solutions were added to a 96-well plate to make final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 μg/mL, and then cultured at 30 °C. After 24 h of culture, 15 μL of 0.2% TTC was added to each well. After being cultured at 37 °C for 4 h with anhydrous ethanol as the control group, color changes were observed. The experiment was repeated three times, and the average value was taken to determine the minimum inhibitory concentration (MIC).

Data Analysis

The changes in the number of algal cells in each group were compared using Excel Chart, and the changing trend was analyzed. Statistical analysis was carried out using GraphPad Prism8.0 software.

Results

Identification of the Eight Chemicals Extracted from J stipulacea

Compound I was identified as palmitic acid, compound II as sitosterol, compound III as betulinic acid, compound IV as gallic acid, compound V as ursolic acid, compound VI as quercetin 3-O-rhamnoside, compound VII as kaempferol, and compound VIII as luteolin. The contents of the eight compounds, determined by HPLC, were luteolin 0.02%, kaempferol 0.02%, and ursolic acid 0.01%. Three compounds with effective algal inhibition were selected: ursolic acid, kaempferol, and luteolin; gallic acid also had a certain bacteriostatic effect. In the ethyl acetate extract, the content of luteolin was 1.54%, the content of kaempferol 1.21%, the content of ursolic acid 0.09%, and the content of gallic acid 1.67%. According to the repeatability, the relative standard deviation (RSD) of luteolin was 2.27%, kaempferol 2.86%, and ursolic acid 2.92%. The linearity and repeatability of the quantitative analysis were good.

Effect of J stipulacea Extract on M aeruginosa

In the visible light range, M aeruginosa and Anabaena had an absorption peak at 680 nm, and the naked algae at 761 nm. Therefore, 680 nm was selected as the most suitable absorption wavelength for determining the biomass of M aeruginosa and Anabaena, and 761 nm for Euglena (Figure S2). With an increase in algal cells, the optical density value increased correspondingly, and there was a significant positive linear correlation between the optical density value of the three types of algal cells and their biomass. According to the repeatability, the corresponding algal biomass could be expressed by the optical density value. Figure 1 shows that the inhibitory effect of allelochemicals from J stipulacea on M aeruginosa increased with an increase in contact time, and the inhibitory effect of the high concentration group was more obvious. At a high concentration of 5-10 g/L, the growth of M aeruginosa was promoted at first and then inhibited. After administration of the highest concentration of 25 g/L, most M aeruginosa cells died, as observed under both the microscope and the naked eye, but, due to the color of the J stipulacea extract, the linear relationship between the absorbance and biomass was disturbed. After exposure to a high concentration of 10 g/L for 96 h, the inhibition rate was as high as 59%. The inhibition rate for M aeruginosa at a low concentration of 1 g/L was about 10%, but this increased slowly with time. This trend of low-dose promotion and high-dose inhibition was reported in the previous study.

Effect of Three Standard Allelochemicals from J stipulacea on the Growth of M aeruginosa, Anabaena, and Euglena

Figure 2 shows the inhibitory effect of ursolic acid, kaempferol, and luteolin on the growth of M aeruginosa. At a concentration of 1.25-10 mg/L, the three drugs had almost no effect on growth, but the high concentration group had a more obvious inhibitory effect with the increase in contact time. When the concentration of kaempferol and luteolin exceeded 20 mg/L, their inhibitory effect on M aeruginosa increased with time. When the concentration of the three drugs was 50 mg/L, the inhibition rate of M
M. aeruginosa was more than 20% after 4 days of administration and their growth was most inhibited. In contrast, different concentrations of erythromycin had inhibitory effects on M. aeruginosa, and the inhibition rate increased with time; the inhibition rate was more than 40% after 96 h.

Figure 3 shows that different concentrations of ursolic acid, kaempferol, and luteolin had inhibitory effects on Anabaena, and with the increase in concentration, the inhibitory effect was more obvious. When the concentration of kaempferol and luteolin was 50 mg/L, a promoting effect was observed after 24 h of exposure. This may be because the high concentration of drugs increased the activity of the cell enzymes, changed the permeability of the algal cell membrane, and made it easier for the algal cells to absorb nutrients from the solution, thus promoting algal growth. After 48 h of exposure, the inhibition rate increased with time. The inhibition rates of ursolic acid, kaempferol, and luteolin on Anabaena after 96 h were 74.8%, 69.2%, and 70.7%, respectively. In contrast, the inhibition rate of erythromycin at 50 mg/L reached more than 50% after 24 h, but, with the extension of time, the inhibitory effects of different concentrations of erythromycin on Anabaena decreased.

Compared with M. aeruginosa and Anabaena, ursolic acid, kaempferol, and luteolin had a lesser inhibitory effect on Euglena. The maximum effect was observed at 20 mg/L at 96 h, and the maximum inhibitory rate was no more than 20%. As shown in Figure 4, after treatment with different concentrations of ursolic acid and kaempferol, the growth of Euglena was inhibited at low concentrations and promoted at high concentrations, while the concentration of 50 mg/L could stimulate the growth of naked algae. In contrast, when the concentration of erythromycin was higher than 10 mg/mL, its inhibitory effect on Euglena was more significant.

**MIC Determination of Luteolin and Florfenicol on S. iniae and Gallic Acid on A. hydrophila**

Triphenyltetrazolium chloride (TTC) is a commonly used live bacterial dye whose oxidation state is colorless. Luteolin had no bacteriostatic effect on S. iniae in the concentration range of 7.81-500 μg/mL, and only showed an inhibitory effect at 1000 μg/mL. Gallic acid had no inhibitory effect on S. iniae in the concentration range of 7.81-500 μg/mL, but began to inhibit at 1000 μg/mL. Florfenicol inhibited S. iniae in the concentration range of 7.81-1000 μg/mL. Gallic acid had no inhibitory effect on A. hydrophila in the concentration range of 7.81-500 μg/mL, but began to show an inhibitory effect at 1000 μg/mL.

**Discussion**

As one of the main techniques of in situ remediations of water, such as a floating bed of plants, J. stipulacea is widely used in...
sewage treatment. *M. aeruginosa* and *Anabaena* are both cyanobacteria, which play a vital role in the food chain as primary producers, and small changes in their populations may affect the ecological balance. The frequent occurrence of cyanobacterial blooms, such as *Microcystis*, seriously affects the quality of drinking water. In particular, microcystins, mainly those produced by *M. aeruginosa*, may cause severe liver damage in humans. Plant allelochemicals can inhibit diseases, pests, and weeds. In this experiment, three kinds of algae were inhibited by allelochemicals extracted from *J. stipulacea*. The inhibitory effect of these allelochemicals on *M. aeruginosa* increased with time, with an inhibition rate of 59% at 10 g/L. A previous study showed that the relative inhibition rate could reach 50%-95% within 4-7 days, which was similar to our experimental results. According to the published results, in the later stage of culture, the inhibition rates at all concentrations showed a downward trend, indicating that the inhibitory effect of the chemicals on the growth of algal cells gradually weakened. This may be related to their degradation or transformation. Luteolin and the other three reference materials displayed inhibitory effects on all three algae, while the *J. stipulacea* extract only had an inhibitory effect on *M. aeruginosa*, which may be because the extract contains antagonistic substances to inhibit algae. The combinations of 35 µg/mL luteolin-5-O-β-d-glucoside and 5 µg/mL verbenoside, and 25 µg/mL luteolin-5-O-β-d-glucoside and 10 µg/mL verbenoside had the strongest antagonistic effects. The antiedematogenic and antiplasmodial activities of betulinic acid, and the antioxidant activity of quercetin 3-O-rhamnoside have been reported in medical herbs, but showed no significant effect in the present study.

Ursolic acid, kaempferol, and luteolin had almost no effect on the growth of *M. aeruginosa* when their concentrations were less than 10 mg/L. When their concentrations were lower than 20 mg/L, the inhibition rate of *M. aeruginosa* increased with time, and when the concentrations were greater than 50 mg/L, the inhibition rate was more than 20% after 96 h; the inhibition rates for luteolin and ursolic acid were 36% and 42%, respectively. Some researchers have also studied the inhibitory effects of luteolin on *M. aeruginosa*, and their results showed that the growth inhibition rate of *M. aeruginosa* generally increased with an increase in the luteolin dose; however, the growth inhibition increased at first and then tended to stabilize or decrease. However, the inhibition of ursolic acid,
kaempferol, and luteolin on *Anabaena* increased with the increase in concentration, and the inhibition rates reached 74.8%, 69.2%, and 70.7%, respectively, after 96 h. Compared with the inhibitory effects on *M. aeruginosa* and *Anabaena*, ursolic acid, kaempferol, and luteolin had less inhibitory effects on *Euglena*, and the maximum inhibitory rate was less than 20%, which resulted in a low concentration inhibition and high concentration promotion.

Allelochemicals can probably inhibit the growth of algal cells due to their ability to increase the respiration rate of algal cells and reduce the rate of photosynthesis and chlorophyll a content. Allelochemicals at lower concentrations could increase the activities of peroxidase, superoxide dismutase, and dehydrogenase in *M. aeruginosa*, while high concentrations significantly decrease the activities of these enzymes. Inhibiting the antioxidant enzyme system of *M. aeruginosa* and promoting the degradation of algal chlorophyll may be among the algal inhibition mechanisms of allelochemicals.

*Streptococcus* in farmed fish has become an important problem in many countries, which can cause acute infection in fish, resulting in a mortality rate of more than 50% within 3-7 days. Due to hemorrhagic septicemia syndrome, which is caused by *A. hydrophila* infection, the mortality rates in aquaculture are increasing, thereby causing huge economic losses and reducing food quality. A previous study showed that the biofilm and extracellular protease activities of *A. hydrophila* significantly decreased by 72.1% and 73.3%, respectively, compared with those of the control when treated with *Z. bungeanum* extract (16.00 mg/mL). *Z. bungeanum* extract showed a concentration-dependent inhibitory effect on the quorum sensing of *A. hydrophila*. A previous study also showed that resveratrol, present in *Polygonum cuspidatum* extract, had a significant inhibitory effect on the biofilm formation and hemolytic activity of pathogenic strains of *A. hydrophila* at the minimum inhibitory concentration of ≥32 μg/mL, and this inhibitory effect increased with an increase in dose. In the present study, gallic acid and luteolin showed inhibitory effects on *A. hydrophila* and *S. iniae*, respectively, at 1000 μg/mL. A pharmaceutical composition with a dihydromyricetin and magnolol mass concentration ratio of 1:2 to 1:16 has been previously used to inhibit *A. hydrophila* infection.

In this experiment, different concentrations of erythromycin had inhibitory effects on *M. aeruginosa*, and the inhibition rate reached more than 40% after 96 h, which was higher than that of the *J. stipitacea* allelochemicals. Algal growth was promoted by low concentrations (0.20 mg/L) and inhibited by
high concentrations (5 mg/L). Some experiments have shown that the dual effect of erythromycin on cyanobacteria may have an excitatory effect on them. This effect is a dose-response relationship characterized by low-dose stimulation and high-dose inhibition. The inhibitory effect of high erythromycin concentrations on Microcystis may be due to the fact that its concentration exceeds the tolerance limit of the algal cells, resulting in the rupture and disintegration of their cell structure, zero or negative growth, and cell death in severe cases. The inhibition rate of Anabaena was more than 50% when the concentration of erythromycin was 50 mg/L for 24 h, but the inhibition rate decreased with time. Its inhibition rate was lower than that of J stipulacea extract, and its inhibition effect on Anabaena was not as effective as that on M aeruginosa. According to previous reports, it is possible that M aeruginosa is more sensitive to antibiotics than Anabaena. When the concentration of erythromycin was higher than 10 mg/L, the inhibitory effect of erythromycin on Euglena was significantly higher than that of the J stipulacea allelochemicals.

In the present study, florfenicol inhibited S iniae in the concentration range of 7.81-1000 μg/mL, and its inhibitory effect was greater than that of luteolin. Florfenicol also has a certain inhibitory effect on A hydrophila, and studies have shown that pomegranate extract can enhance the bacteriostatic effect of florfenicol. However, an increase in florfenicol concentration causes serious damage to genetic material. Antibiotics play an important role in animal husbandry and modern agriculture as therapeutic drugs and growth promoters in animal husbandry production and as feed additives in fish farms, as well as preventing damage to crops caused by pathogenic bacteria. The toxicity of erythromycin to algae is usually evaluated by inhibiting algal growth, interfering with photosynthesis, causing oxidative damage, and hindering other metabolic processes. A high concentration (40 mg/L) of erythromycin may significantly inhibit the growth and photosynthesis of M aeruginosa. Florfenicol has also been shown to be effective against various bacterial pathogens. Erythromycin and florfenicol are both antibiotics. In this study, we verified that J stipulacea allelochemicals have positive bacteriostatic and algal-killing effects,
Conclusion
The crude extract of *J. stipulacea* had an inhibitory effect on *M. aeruginosa*, especially in the high concentration group, and the inhibition rate reached 59% after exposure to 10 g/L for 96 h. Ursolic acid, kaempferol, and luteolin had inhibitory effects on *M. aeruginosa*, with inhibition rates of more than 20% after 96 h at 50 mg/L, while the inhibition rates of different concentrations of erythromycin on *M. aeruginosa* reached more than 40% after 96 h. The three standard allelochemicals also had a positive inhibitory effect on *Anabaena*. When the concentration was 50 mg/L, the inhibition rate of *Anabaena* was as high as 74.8%, 69.2%, and 70.7% for ursolic acid, kaempferol, and luteolin, respectively, while the inhibition rate of erythromycin decreased with time. Ursolic acid, kaempferol, and luteolin had no obvious inhibitory effect on *Euglena*, but erythromycin had an obvious inhibitory effect when the concentration was higher than 10 mg/L. Luteolin showed an inhibitory effect on *S. iniae* at 1000 μg/mL, while florfenicol inhibited it in the concentration range of 7.81-1000 μg/mL. Gallic acid began to inhibit *A. hydrophila* at 1000 μg/mL.

Acknowledgements
The authors thank Kristina (English editor for editage) and Lianhong Yin (Dalian Medical University) for providing grammar and spelling check, and giving revision suggestions for the manuscript.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The work was supported by the open project of Agriculture Ministry Key Laboratory of Healthy Freshwater Aquaculture (ZJK202102), China Agriculture Research System of MOF and MARA (No. CARS-46), Special Fund of Fundamental Scientific Research Business Expense for Central Public Research Institutes (No. 2021JBFM19).

Ethical Approval
Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

ORCID iD
Yao Zheng https://orcid.org/0000-0001-5958-6008

Supplemental Material
Supplemental material for this article is available online.

References
1. Jarau M, MacInnes JI, Lumsden JS. Erythromycin and florfenicol treatment of rainbow trout *Oncorhynchus mykiss* (Walbaum) experimentally infected with *Flavobacterium psychrophilum*. *J Fish Dis.* 2019;42(3):325-334. doi: 10.1111/jfd.12944
2. Harikrishnan R, Balasundaram C. Modern trends in *Aeromonas hydrophila* disease management with fish. *Rev Fish Sci.* 2005;13(4):281-320.
3. Bhuvaneswari BCR. Traditional Indian herbal extracts used in vitro against growth of the pathogenic bacteria- *Aeromonas hydrophila*. *Int J Aquacult-Bamid*. 2006;2:89-96.
4. Zhu X, Dao G, Tao Y, Zhan X, Hu H. A review on control of harmful algal blooms by plant-derived allelochemicals. *J Hazard Mater*. 2021;401:123403. doi: 10.1016/j.jhazmat.2020.123403
5. Li B, Yin Y, Kang L, et al. A review: application of allelochemicals in water ecological restoration--algal inhibition. *Chemosphere*. 2021;267:128869. doi: 10.1016/j.chemosphere.2020.128869
6. Zhang B, Guo J, Fang F. Mechanism of phytoallelopathy and algal inhibition. *J Ecol*. 2010;29:1846-1851.
7. Hong Y, Hu H. Study and application of allelopathy and algal inhibition of aquatic plants. *Sci Bull*. 2009;54:287-293.
8. Zheng Y, Hu G, Wu W, et al. Transcriptome analysis of juvenile genetically improved farmed tilapia (*Oreochromis niloticus*) livers by dietary resveratrol supplementation. *Comp Biochem Physiol C Toxicol Pharmacol*. 2019;223:1-8. doi: 10.1016/j.cbpc.2019.04.011
9. Zheng Y, Hu G, Wu W, Qiu L, Bing X, Chen J. Time-dependent gut microbiota analysis of juvenile *Oreochromis niloticus* by dietary supplementation of resveratrol. *Arch Microbiol*. 2020;202(1):43-53. doi: 10.1007/s00203-019-01712-1
10. Wu X, Yang X, Li T, Fang Y. Study on the purification effect of floating plants on eutrophic landscape water. *J Soil Water Conserv*. 2007;21:128-132.
11. Li F, Hu H, Zhong Y, Men Y, Guo M. Effects of reed allelochemicals EMA on the physiological characteristics of *Microcystis aeruginosa*. *Environ Sci China*. 2007;27:377-381.
12. Khan MJ, Gordon R, Varjani S, Vinayak V. Employing newly developed plastic bubble wrap technique for biofuel production from diatoms cultivated in discarded plastic waste. *Sci Total Environ*. 2022;823:153667. doi: 10.1016/j.scitotenv.2022.153667
13. Takahashi T. Routine management of microalgae using autofluorescence from chlorophyll. *Molecules*. 2019;24(24):4441. doi: 10.3390/molecules24244441
14. Liu F, He ZB, Li HY, Liu JS, Yang WD. Inhibition of five natural products from Chinese herbs on the growth of *Chattonella marina*. *Environ Sci Pollut Res Int*. 2016;23(17):17793-17800. doi: 10.1007/s11356-016-6755-5
15. Sierra-Zapata I, Álvarez JC, Romero-Tabarez M, et al. Inducible antibacterial activity in the bacillales by triphenyl tetrazolium chloride. Sci Rep. 2020;10(1):5563. doi: 10.1038/s41598-020-62236-z

16. Brown AR, Ettefagh KA, Todd DA, et al. Bacterial efflux inhibitors are widely distributed in land plants. J Ethnopharmacol. 2021;267:113533. doi: 10.1016/j.jep.2020.113533

17. Paddy MR, Dahquist FW, Drazt EA, Deese AJ. Simultaneous observation of order and dynamics at several defined positions in a single acyl chain using 2H NMR of single acyl chain perdeuterated phosphatidylethanolamines. Biochim. 1985;24:5988-5995. doi: 10.1012/bi00342a045

18. Chandler RF, Hooper SN, Hooper DL, Jamieson WD, Lewis E. Herbal remedies of the Maritime Indians: sterols and triterpenes of Tanacetum vulgare L. (Tansy). Lipoic. 1982;17:102-106. doi: 10.1007/BF02535183

19. Zhu M, Phillipson JD, Greengrass PM, Bowery NG. Chemical and biological investigation of the root bark of Clerodendrum mandarinorum. Planta Med. 1996;62:393-396. doi: 10.1055/s-2006-957923

20. Karaseva TA, Kuts’ VS, Shamrai EF. Galacosamine structure, studied by nuclear magnetic resonance. Ukr Biokhim Zh. 1975;47:514-517.

21. Kriwacki RW, Piter TP. Current aspects of practical two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy: applications to structure elucidation. Pharm Res. 1989;6:531-544. doi: 10.1007/s11095-012-09078-8

22. Muzitano MF, Tinoco LW, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS. The antileishmanial activity assessment of unusual flavonoids from Kalanchoe pinnata. Phytochem. 2006;67:2071-2077. doi: 10.1016/j.phytochem.2006.06.027

23. Markham KR, Geiger H, Jaggy H. Kaempferol-3-O-glucosyl(1-2) rhamnoside from Ginkgo biloba and a reappraisal of other glucos(1-2, 1-3) and 1-4) rhamnoside structures. Phytochem. 1992;31:1099-1101. doi: 10.1016/0031-9422(92)80058-n

24. Post LF, Varma RS. Growth inhibitory effects of bioflavonoids and related compounds on human leukemic CEM-C1 and CEM-C7 cells. Cancer Lett. 1992;67:207-213. doi: 10.1016/0304-3835(92)90145-1

25. He C, Ye J. Studies on the algal control effect of Aureus tatarinowii. Acta Ecol. 1999;19(3):754-758.

26. Valitato K, Kruglova A, Mikola A, Vahala R. Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. Int J Hyg Environ Health. 2017;220:558-569. doi: 10.1016/j.ijih.2017.02.003

27. Ueno Y, Nagata S, Tsutsumi T, et al. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis. 1996;17:1317-1321. doi: 10.1093/carcin/17.6.1317

28. Codd GA, Morrison LF, Metcalf JS. Cyanobacterial toxins: risk management for health protection. Toxicol Appl Pharmacol. 2005;203:264-272. doi: 10.1016/j.taap.2004.02.016

29. Wu X, Wu H, Ye J. Influences of allelochemical extracted from Jussiaea stipulacea Ohwi on growth, microcystins production, and release of Microcystis aeruginosa. Oceans Lakes. 2014;783-788.

30. Frum Y, Viljoen AM, Heerden FRV. Verbasconside and luteolin-5-O-p-d-glucoside isolated from Halleria lucida L. exhibit antagonistic anti-oxidant properties in vitro. South African J Botany. 2007;73:583-587. doi: 10.1016/j.sajb.2007.05.006

31. Alves VG, da Rosa EA, de Arruda LL, et al. Acute toxicity, anti-oxidemagenic activity, and chemical constituents of Palicourea rigida Kunth. Z Natuforsch C J Biosci. 2016;71:39-43. doi: 10.1515/znc-2015-0036

32. Su Q, Dalal S, Goetz M, Cassera MB, Kingston DG. Antiplasmodial phloroglucinol derivatives from Synaptia glomulifera. Bioorg Med Chem. 2016;24:2544-2548. doi: 10.1016/j.bmc.2016.04.020

33. Ramos AS, Mar JM, da Silva LS, et al. Pedra-ume caá fruit: an Amazon cherry rich in phenolic compounds with antilgycant and antioxidant properties. Food Res Int. 2019;126:674-683. doi: 10.1016/j.foodres.2019.05.042

34. Adeniyi O, Baptista R, Bhowmick S, et al. Isolation and characterisation of quercitrin as a potent anti-sickle cell anaemia agent from Alchornea cordifolia. J Clin Med. 2022;11:2177. doi: 10.3390/jcm11082177

35. Li J, Hu J, Cao L, Yuan Y. Growth, physiological responses and microcystin-production/-release dynamics of Microcystis aeruginosa exposed to various luteolin doses. Environ Sci Pollution. 2019;16;110540. doi: 10.1016/j.ecison.2020.110540

36. Qian H, Xu X, Chen W, et al. Growth inhibitory effects of bacteria for aerobic Microcystis aeruginosa. Int J Hyg Environ Health. 2017;211:119-123. doi: 10.1016/j.ijheh.2017.10.005

37. Dong J, Ding H, Liu Y, et al. Magnolol protects channel catfish from Aeromonas hydrophila infection via inhibiting the expression of aerolysin. Vet Microbiol. 2017;211:119-123. doi: 10.1016/j. vetmic.2017.10.005

38. Shi et al. Sci Rep. 2020;10(1):5563. doi: 10.1038/s41598-020-62236-z
46. Halling-Sørensen B. Algal toxicity of antibacterial agents used in-intensive farming. *Chemosphere*. 2000;40(7):731-739. doi: 10.1016/s0045-6535(99)00445-2

47. Halling-Sørensen B, Lutzhøft HCH, Andersen HR, Ingerslev F. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. *J Antimicrob Chemother*. 2000;46(S1):53-58.

48. Robinson AA, Belden JB, Lydy MJ. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environ Toxicol Chem*. 2005;24(2):423-430. doi: 10.1897/04-210r.1

49. Zhou H, Gai C, Ye G, et al. *Aeromonas hydrophila*, an emerging causative agent of freshwater-farmed whiteleg shrimp *Litopenaeus vannamei*. *Microorganisms*. 2019;7:450. doi: 10.3390/microorganisms7100450

50. El-Sayed A-KI, Soltan MA, Radwan HA, Mohamed MG. Effect of oxytetracycline and florfenicol on the cytogenetic picture of Nile Tilapia. *J Appl Biol Sci*. 2003;7:102-106.

51. Samuelsen OB. Pharmacokinetics of quinolones in fish: a review. *Aquaculture*. 2006;255:55-75. doi: 10.1016/j.aquaculture.2005.12.008

52. Wan J, Guo P, Peng X, Wen K. Effect of erythromycin exposure on the growth, antioxidant system and photosynthesis of *Microcystis flos-aquae*. *J Hazard Mater*. 2015;283:778-786. doi: 10.1016/j.jhazmat.2014.10.026