EFFECTS OF FOUR TRADITIONAL CHINESE MEDICINES ON THE GROWTH OF *Heterosigma akashiwo*

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Introduction:

Aquaculture has become integral to global efforts to meet the growing demands for food security and sustainable resource management. However, due to the harmful algal blooms (HABs), a great number of fish deaths, result in significant economic losses in fish farming.

The increasing reliance on aquaculture comes with the need for innovative approaches to enhance productivity, improve aquaculture health, and minimize environmental impacts. In comparison to other methods of reducing HABs, such as the use of algicides, traditional Chinese medicines (TCMs) offer a potentially safer alternative with fewer negative effects on the environment and human health. Four TCMs, namely *Astragali Radix* (AR), *Coptidis Rhizoma* (CR), *Lonicera Japonica Flos* (LJF), and *Poria* (P), were selected to explore their effects on the growth of harmful algae, *Heterosigma akashiwo* (strain GY-H24).

Objectives:

To investigate the inhibitory effects of Chinese medicine on the growth of harmful algae, *Heterosigma akashiwo*.

Methods:

Preparation of Chinese Medicine

The four selected Chinese medicines (AR, CR, LJF, P) were cut and mashed into powder and passed through a 60-mesh strainer separately. A 100 mg/mL stock solution was prepared by soaking the powder with di-water for 5 hours at 25°C. The extracted solutions were centrifuged and filtered with a 0.2 um sterilized syringe filter before use. Dilute the stock solution into different concentrations with di-water and the filtered Chinese medicine extracts were kept at 4°C before use.

Inhibitory effects of Chinese medicine on the growth of harmful algae, *Heterosigma akashiwo*

The algal cultures of *Heterosigma akashiwo* (GY-H24), purchased from Shanghai Guangyu Biological Technology Co., Ltd, were grown in an L1 medium prepared with sterile-filtered seawater. The algae were incubated at 25°C, with a 12-hour light-dark cycle in an illuminating incubator at 3,000 lux. When 10,000 cells/ml of *Heterosigma akashiwo* are reached, the cells will be treated with either 0, 1, or 10 mg/mL of each of the four Chinese medicines for acute treatment for 6 hours, or sub-acute treatment for 24 hours, respectively. The samples were counted at 6 hours, and 24 hours using a Sedgewick Rafter Counting Chamber under a light microscope. The results were analyzed by one-way ANOVA, followed by Dunnett's multiple comparisons post-test.

Results:

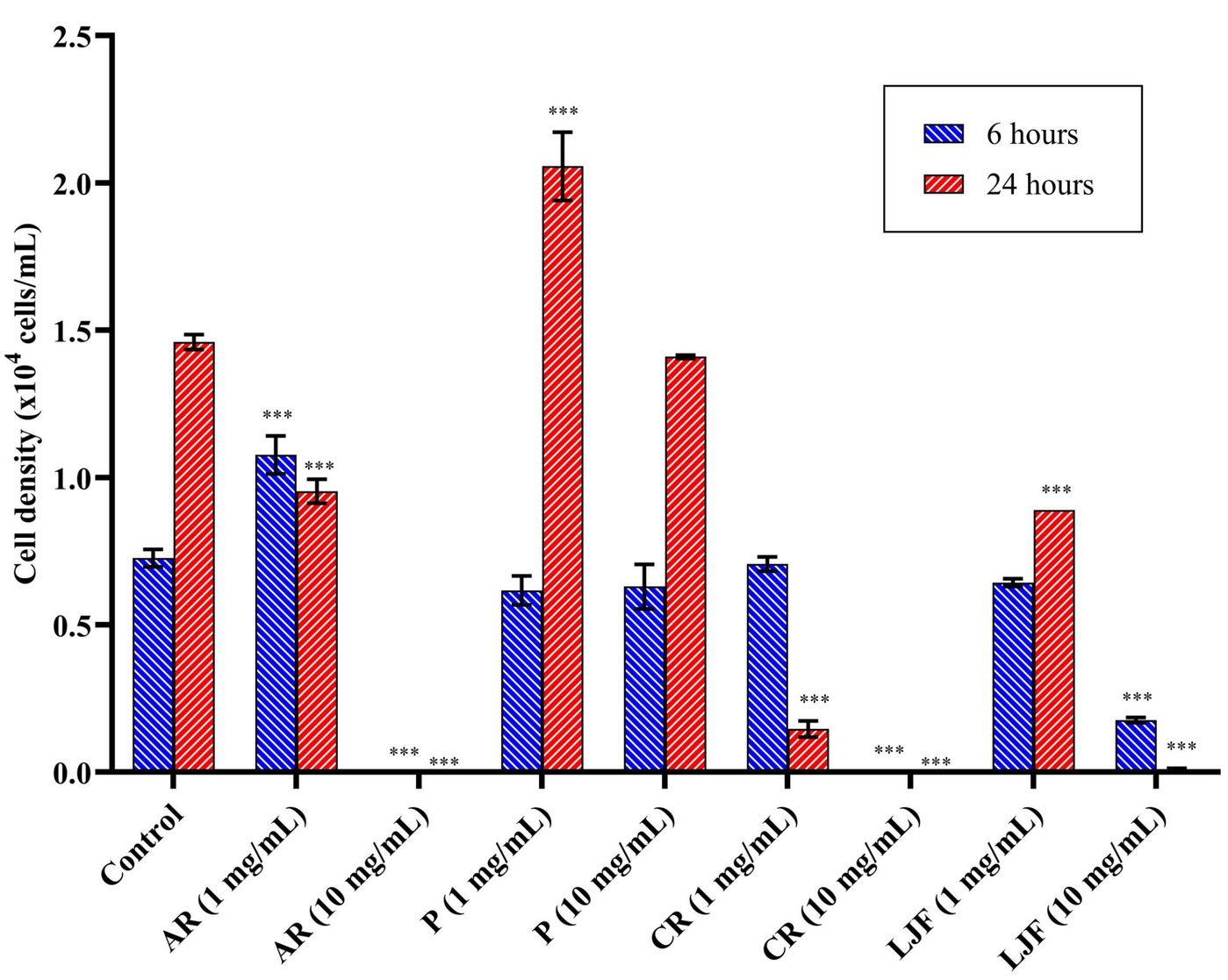

Figure 1 Effect of the four TCM extracts on the growth of *Heterosigma akashiwo* at 0, 1 and 10 mg/mL with different treatment durations, i.e. 6 hours or 24 hours. Mean with SEM of three replicates were shown above. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ indicate the significant difference compare with the control.

Table 1 Summary of effects of the four Chinese Medicine extracts on *Heterosigma alkashiwo* with different treatment durations, i.e. 6 hours or 24 hours. LC50 is the concentration needed to kill 50% population of the algae cells (n = 3).

Chinese medicines	LC50 (mg/mL)	
	6-hour	24-hour
AR	6.464	1.063
P	No inhibition	
CR	1.351	0.499
LJF	4.394	1.192

Conclusion:

Three TCMs, including AR, CR, and LJF, showed prominent inhibitory effects. In contrast, P appeared to have a growth-stimulating effect (Figure 1).

At high concentration (10 mg/mL), AR and CR performed satisfactory results on inhibiting algal growth at both 6- and 24-hour treatment, and LJF performed better in 24-hour treatment than in 6-hour treatment. At low concentration (1 mg/mL), AR, CR, and LJF had an inhibitory effect only at 24-hour treatment, by comparing the LC50 in 6- and 24-hour treatment, CR had a better inhibitory effect than AR and LJF. For P, no inhibitory effect was found, on the contrary, it improved the algal growth at low concentration (1 mg/mL) under 24-hour treatment. Further studies were suggested for investigating the underlying mechanisms for the effects of Chinese medicines on the growth of *Heterosigma akashiwo*.

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