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THE POTENTIAL OF DRIED AND FRESH EXTRACTS OF SANSEVIERIA TRIFASCIATA TO MITIGATE ALEXANDRIUM TAMIYAVANICHII, A TOXIC DINOFLAGELLATE

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ABSTRACT

Dinoflagellate is the second largest group of phytoplankton in the marine environment and many of the species have been recognized as toxic species. The high abundance of this species can cause a lot of problems to human health, marine organisms especially caged fish and the natural environment. In Malaysia, several species of Alexandrium have been reported (RE) were determined for each concentration tested. The extracts were also tested on brine shrimp to see the possible harmful effect. Results indicate that both extraction methods yielded positive results on A. tamiyavanichii whereby the crude extracts managed to mitigate the species. The LC50 of brine shrimp recorded were 30 mg/ml for fresh plant extract and 70 mg/mL for dried plant extracts. This indicates the possibility of the extractions to be used to mitigate harmful algal bloom (HAB) particularly Alexandrium and provide an alternative way to the relevant agencies to minimize the impact of HAB

KEYWORDS

Alexandrium tamiyavanichii, Sansevieria trifasciata, distilled water crude extract, removal efficiency.

1. INTRODUCTION

According to a study, medicinal plants were widely used during the Harmful algal bloom (HAB) is an aquatic phenomenon that occur when there is discoloration of water caused by bloom of microscopic algae whether in marine or freshwater environment. Occurrence of the bloom can cause enormous lost in aquaculture industries, economic and also harmful to human and animal health. For decade, this problem is globally occurred. Based on a study, in Malaysia, several cases have been reported relate to contaminated shellfish by toxic algae species involving Alexandrium [1]. Many studies have been conducted to find the best method for HABs mitigation that is feasible, cost-effective and has minimum impacts to the environment [2]. Most popular method is applying clay to the infected area. This method is very famous and has been applied in several countries such as Korea, Japan, China, Thailand and Malaysia [3]. Other than that, biological approaches like algicidal bacteria (*Micrococcus luteus*) also have been used to reduce the damaged caused by harmful algae [4]. According to a researcher, all of these methods produced positive results, and some showed specific effect on certain species [5]. However, some of the methods mentioned will cause pollution to the environment and affect others marine life. Cells of *A. tamiyavanichii* were isolated from samples collected from Kuantan Port. The cultures were grown at 26ºC with 12:12 L: D (light and dark) cycle and maintained in ES-DK medium enriched with f/2 vitamin. The light intensity used was 1000 lux. positive results and some showed specific effect on certain species [5]. However, some of the methods mentioned will cause pollution to the environment and affect others marine life dried and fresh plants. Different concentrations of crude extracts (0.001, 0.01, 0.1, 0.5, 1, 1.5, 2 and 2.5 mg/mL) were tested on *A. tamiyavanichii* within 24 hours the removal efficiencies.

Nowadays, the use of allelopathy for HABs control has become very popular among researchers due to low cost and environmental friendly [6]. Based on a study, in Malaysia, study on HABs control still at initial stage. Looking at the impact of HAB on human health and economy, it is an urgent need to identify potential way to mitigate HAB species [1,7]. An ornamental plant which is Sansevieria trifasciata or known as snake plant was chosen for the study as the preliminary study on mitigation of HAB showed a positive result [unpublished data]. This plant is popular in traditional medicine and being used in many studies due to its benefits in medicinal, fodder, soil conservation and fibre [8]. Therefore, in this study, crude extract of S. trifasciata was used to mitigate a toxic dinoflagellate, A. tamiyavanichii isolated from Kuantan, Pahang. Besides, the LC50 of the crude extracts on brine shrimp were determined to represent its effect on other marine organisms. Through this study, an alternative way for HABs mitigation particularly A. tamiyavanichii can be suggested. The outcomes of the study are expected to contribute significantly on mitigation of HAB particularly in Malaysia.

2. EXPERIMENTAL

2.1 A. *tamiyavanichii* culture

Cells of *A. tamiyavanichii* were isolated from samples collected from Kuantan Port. The cultures were grown at 26ºC with 12:12 L: D (light and dark) cycle and maintained in ES-DK medium enriched with f/2 vitamin. The light intensity used was 1000 lux.

2.2 Extraction of *S. trifasciata*

Plants were washed, weighted and cut. To obtain dried sample, plants were dried in the oven at 40ºC until constant weight were obtained. Then, both samples (fresh and dried) were grinded using mixer before soaked in distilled water and heated in water bath at 60ºC for 8 hours. Then, crude extracts were filtered before placed in -80ºC freezer and finally undergo freeze drying.

2.3 Test of extracts on A. tamiyavanichii

These crude extracts were tested on A. tamiyavanichii at different concentrations (0.001, 0.01, 0.1, 0.5, 1, 1.5, 2.0 and 2.5 mg/mL). The effects

of each concentration of the crude extracts were determined at time interval of 0, 2.5, 5, 10 and 24 hours. The cell density tested was approximately 1000 to 2000 cells/mL. Tests were conducted in 60×10 mm Petri dish with 3 replicates and 1 control for each concentration. pH of the medium after mixed with the crude extracts were measured for each concentration. Numbers of cells were counted twice under light microscopy at total magnification of 100× using Sedgewick rafter cell for each concentration and time tested. Finally, the effects of the crude extracts on the growth of A. tamiyavanichii were determined based on removal efficiency (RE). The formula used as follows. The higher removal efficiency means that the extract is more effective in removing the cells.
RE = [(initial cell concentration - sample cell concentration)/initial cell concentration] x 100%

2.4 Toxicity test

Brine shrimp usually being used in toxicity test because its lethality bioassay was rapid, easily to get, simple and cheap. The cysts were hatched in sterile seawater for 24 hours before tested with different concentrations of crude extracts which were 10, 50, 100 and 500 mg/mL. The tests were conducted in 60×10 mm petri dish with 3 replicates and 1 control. There were 10 tails of brine shrimp in each Petri dish. LC50 at the 95% confidence limits were obtained by plotted survivors' percentages with concentrations tested.

2.5 Statistical analysis

Results were analysed by one-way ANOVA using SPSS 20.0 to see if there were significant differences between concentrations and times tested for both extracts. Correlation analysis was performed to see the relationship of REs with concentrations and time exposed.

3. RESULTS AND DISCUSSION

3.1 Test of extracts on A. tamiyavanichii

Figures 1 and 2 showed the results of removal efficiency of fresh and dried plant extracts on A. tamiyavanichii. The highest result of removal efficiency of fresh plant extract on A. tamiyavanichii was recorded at concentration of 2 mg/mL which was 88.1% at 0 hours whereas the lowest removal efficiency was 26.85% that tested with 0.001 mg/mL concentration at 2.5 hours (Figure 1). The pHs for both concentrations after mixed the extracts with medium containing A. tamiyavanichii were 5.96 and 8.27, respectively (Table 1).

The removal efficiency of dried plant extract on A. tamiyavanichii showed that the algae exposed to 1.5 mg/mL concentration had the highest result which was 78.24% at 5 hours while the lowest removal efficiency was 23.28% tested with 0.1 mg/mL plant crude extract at 24 hours (Figure 2). The pHs recorded were 6.27 and 8.28 for concentration 1.5 and 0.1 mg/mL after mixed the extract with media containing A. tamiyavanichii (Table 1). Both extracts showed significant differences ($p<0.05$) of removal efficiency between times and concentrations for all experiment conducted. Results showed that A. tamiyavanichii reacts differently on different concentrations of both extracts tested. Statistical analysis showed a positive correlation (r = 0.76, r = 0.67) extract at 24 hours (Figure 2). The pHs recorded were 6.27 and 8.28 for concentration 1.5 and 0.1 mg/mL after mixed the extract with media containing A. tamiyavanichii (Table 1). Both extracts showed significant differences ($p<0.05$) of removal efficiency between times and concentrations for all experiment conducted.

Results showed that A. tamiyavanichii reacts differently on different concentrations of both extracts tested. Statistical analysis showed a positive correlation (r = 0.76, r = 0.67) between removal efficiency and concentration for both crude extracts tested. However, between removal efficiency and time, negative correlations (r = -0.29, r = -0.44) were obtained. Previous study on harmful algae, P. globosa and P. donghaiense using Chinese traditional herbs and herb-modified clay have showed that the inhibitory rate of algae increased as the concentration applied increased [9]. Result obtained also showed that as the concentration increased, pH decreased, and removal efficiency increased (Table 1). The dropped of medium pH after the extracts were added need to be given attention. This is because different species has different tolerant towards pH [10]. This is to ensure that high RE recorded is due to the effect of crude extracts tested and not change in pH of the medium.

Table 1: Result summary of plant extracts

Fresh plant extract			Dried plant extract	
	highest	lowest	highest	lowest
Concentration (mg/mL)	2	0.001	1.5	0.1
RE (%)	88.1	26.85	78.24	23.28
Time (hour)	0	2.5	5	24
pH	5.96	8.27	6.27	8.28
LC50		30 mg/mL		70 mg/mL

Both extracts showed significant differences ($p<0.05$) of removal efficiency between times and concentrations for all experiment conducted. Results showed that *A. tamiyavanichii* reacts differently on different concentrations of both extracts tested. Statistical analysis showed a positive correlation (r = 0.76, r = 0.67)

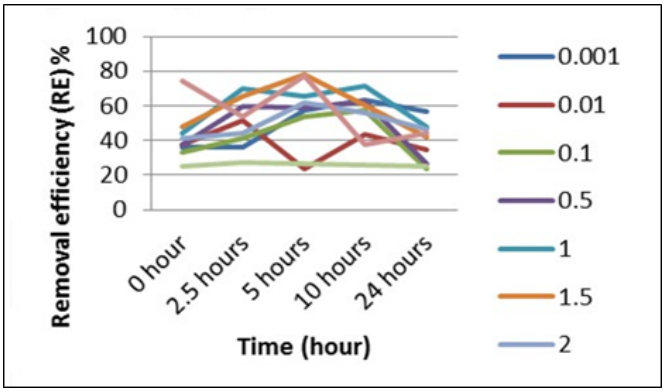

Figure 1: Removal efficiencies (RE) (%) of fresh plant extracts on A. tamiyavanichii

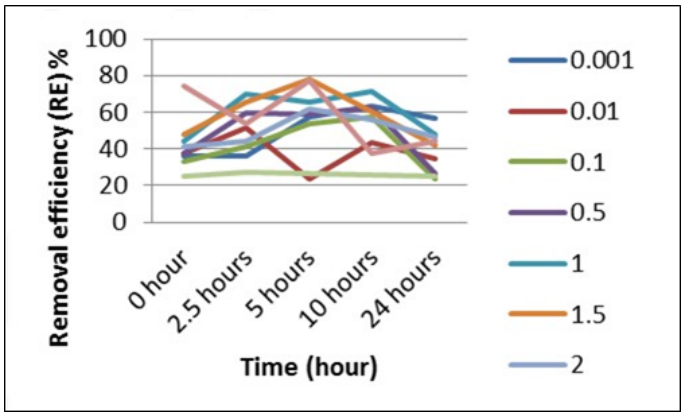

Figure 2: Removal efficiencies (RE) (%) of dried plant extracts on A. tamiyavanichii

3.2 Toxicity test

Figure 3 showed the results of LC_{50} of distilled water fresh and dried plant extracts on brine shrimp (artemia). The LC_{50} of distilled water fresh plant extract on artemia was lower i.e. 30mg/mL compared to dried plant extract i.e. 70 mg/mL (Figure 3). From the toxicity result, high concentrations of extracts were needed to kill the brine shrimp. This study helps to prove that the extracts were suitable to be applied at the field as the concentration needed to kill
harmful algae will not affect others marine life.

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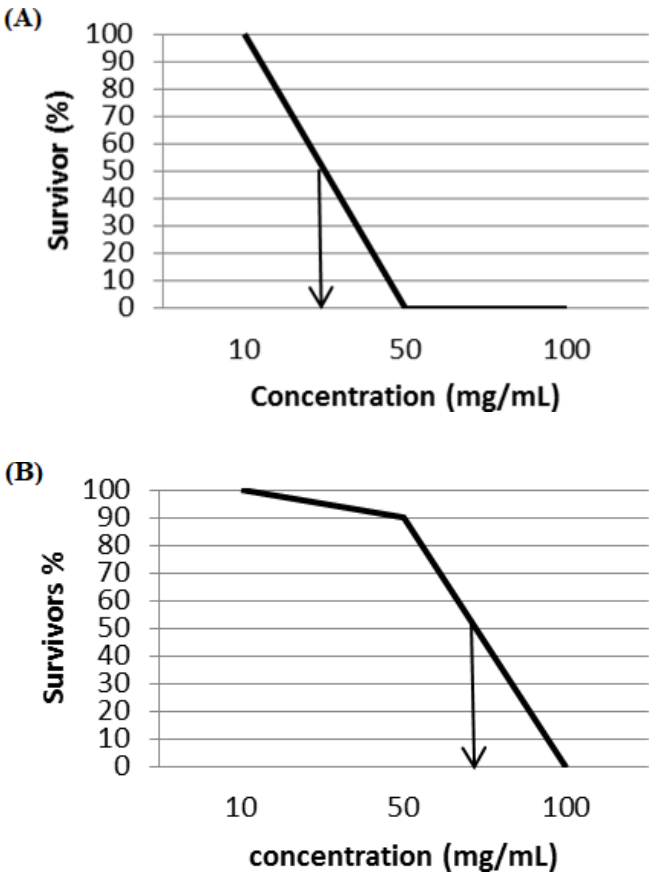

Figure 3: LC50 of fresh plant extract (A) and dried plant extract (B) on artemia

4. CONCLUSION

Results of removal efficiencies showed different effectiveness between both crude extracts (fresh and dried plant extract of *S. trifasciata)* on *A. tamiyavanichii*. However, overall results showed that the pH influenced the results by increasing the removal efficiency. Therefore, high concentration of crude extracts is not recommended especially for toxic species. This is because the cells will lyse and toxin will be released to the environment posing other secondary effect. Nevertheless, this study indicates that ornamental plant, *S. trifasciata* has the potential to inhibit the growth of *A. tamiyavanichii* but further study is needed to fine tune the result in order to find the suitable concentration needed to mitigate HAB species.

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