Current Applied Science and Technology Vol. 22 No. 2 (March-April 2022)

Research article

Allelopathic Potential of Subfractions from Aqueous Extract of *Spirulina platensis* and Denatured C-phycocyanin

Patchanee Charoenying [1]*, Chamroon Laosinwattana [2] and Nawasit Chotsaeng [1]

[1]*Department of Chemistry, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*
[2]*Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*

Received: 21 December 2020, Revised: 15 July 2021, Accepted: 27 July 2021

DOI: 10.55003/cast.2022.02.22.007

Keywords

allelopathic potential;

Spirulina platensis;

denatured C-phycocyanin;

aqueous extract

Abstract

In this laboratory bioassay, crude aqueous extract of *Spirulina platensis* (Nordst) Geitl. was extracted with 90% aqueous ethanol and 80% aqueous ethanol to achieve three subfractions, green powder (F1), pale-green powder (F2) and deep-blue powder (F3). Each of these subfractions was evaluated for allelopathic activity on Chinese amaranth (*Amaranthus tricolor* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.). The plant seeds were germinated in vials with three subfractions (250-2000 mg/l concentrations) and distilled water was used as a negative control. The results indicated that subfraction F3 had the highest inhibitory effect on both of the tested plants. All subfractions had an absorbance ratio (A620/A280) lower than the original aqueous extract, suggesting that a denaturation event of the C-phycocyanin (C-PC) component had occurred during the extraction process. After heating a commercial C-PC solution at 90°C for 3 h, the absorbance ratio changed to 0.46, and likewise allelopathic assay revealed that the decolorized C-PC solution retained its allelopathic potential on the tested plants. To confirm these findings, *S. platensis* powder was extracted with distilled water at 90°C for 3 h and tested for its allelopathic potential using standard Petri dish assay at concentrations of 0.625-5%. The results showed that the hot aqueous extract had an absorbance ratio of 0.38, and its allelopathic activities were similar to those of the aqueous extract. Furthermore, it was found that although C-PC in aqueous extractions denatured, it still retained its allelopathic potential on the tested plants.

*Corresponding author: Tel.: (+66)-2329-8400 (ext. 6241)
E-mail: patchanee.ch@kmitl.ac.th

1. Introduction

Weeds are major problem in crop production worldwide and the main weed control method used is chemical control. Although this method is the most common, the prolonged use of synthetic chemicals has resulted in weeds becoming more resistant to herbicides. Moreover, these herbicides can be toxic to humans and harmful to the environment. Allelopathy is defined as the direct or indirect detrimental or beneficial effects of one plant (including microorganisms) on the germination, growth, or development of other plants through the production of chemicals (allelochemicals) that are released into the environment [1, 2]. This effect plays a significant role in agroecosystems as it modulates the quality and quantity of produce and commercially important crops [3, 4]. Such effects have been applied in agricultural science in areas such as weed management, and continue to be attractive candidates for crop control since they are naturally occurring and are considered as environmentally friendly. Moreover, allelochemicals are renewable and easily degradable. Not only has the allelopathic effect been recognized in terrestrial plants, it has also been seen in microorganisms, such as algae [5-7]. Previously, some cyanobacteria were reported to have harmful effects on the germination and growth of other species. For example, *Nostoc* 31 showed allelopathic activity against cyanobacteria *Anabaena* 7120 [8]. The photosynthetic cyanobacterium *Spirulina platensis* displays many biological activities including antioxidant, anti-inflammatory, and anticancer effects. It is often used to produce biologically active food additives and to treat certain diseases [9-11]. Our preliminary study revealed that an aqueous extract of *S. platensis* was able to affect seed germination and seedling growth of tested plants. We further studied the allelopathic effect of biologically active protein pigment, C-phycocyanin (C-PC), which is the major component of the *S. platensis* aqueous extract. Our results showed that C-PC was able to significantly inhibit properties of the tested plants [12]. In order to better understand this inhibitory phenomenon and specifically determine the active fraction and its components, a series of extraction were made from the crude aqueous extracts and the allelopathic effects of the hot aqueous extract and degraded C-PC were investigated.

2. Materials and Methods

2.1 Reagents and tested plants

Dried biomass *Spirulina platensis* was obtained from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL). Algal cultures were grown in Zarrouk medium [13] and maintained in a 0.03% CO_2 atmosphere at 25°C and pH 10.5. The cultivation flasks were illuminated under 400 $\mu molm^{-2}s^{-1}$ light intensity. Cells were harvested at the late exponential phase by centrifugation and dried in an oven at 40°C. Analytical grade C-phycocyanin was purchased from Sigma-Aldrich, (Saint Louis, MO, USA). All other chemicals used were of reagent grade. The seeds for barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) were randomly collected from rice fields in Phisanulok Province, Thailand in August 2018, and Chinese amaranth (*Amaranthus tricolor* L.) was purchased from Thai Seed & Agriculture Co., Ltd. company, Bangkok, Thailand. The germination rate of the test seeds was > 80%.

2.2 Preparation of subfractions of *S. platensis* aqueous extracts

Dried *S. platensis* (5 g) was soaked in 95 ml of distilled water in a 125 ml Erlenmeyer flask with stirring for 10 min at room temperature. The flask was sealed with Parafilm and stored in a refrigerator at 4°C for 24 h. Afterwards, the mixture was centrifuged at 5000 rpm for 20 min to

obtain 5% w/v of aqueous extract. Fifty milliliters of the extract were dehydrated under a vacuum (Freeze Dryer and AdVantage data center wizard 2.0, Virtis, NY, USA) to obtain a blue-green powder. Next, extraction with 90% (v/v) aqueous ethanol was performed on the powder, and the resulting extract was centrifuged at 5000 rpm for 20 min, and a green supernatant and a blue precipitate were obtained. A further extraction with 80% (v/v) aqueous ethanol was performed on the blue precipitate, and this extract was centrifuged as described in the previous experiment, giving a blue precipitate and a light-green supernatant. The green and the light-green supernatants were then evaporated to dryness using a rotary vacuum evaporator at 40ºC. A green powder (F1), a pale-green powder (F2), and a blue precipitate were obtained [14]. The deep-blue precipitate was dried under N_2 (g) flow to yield a deep-blue powder (F3). The extraction procedure is shown in Figure 1.

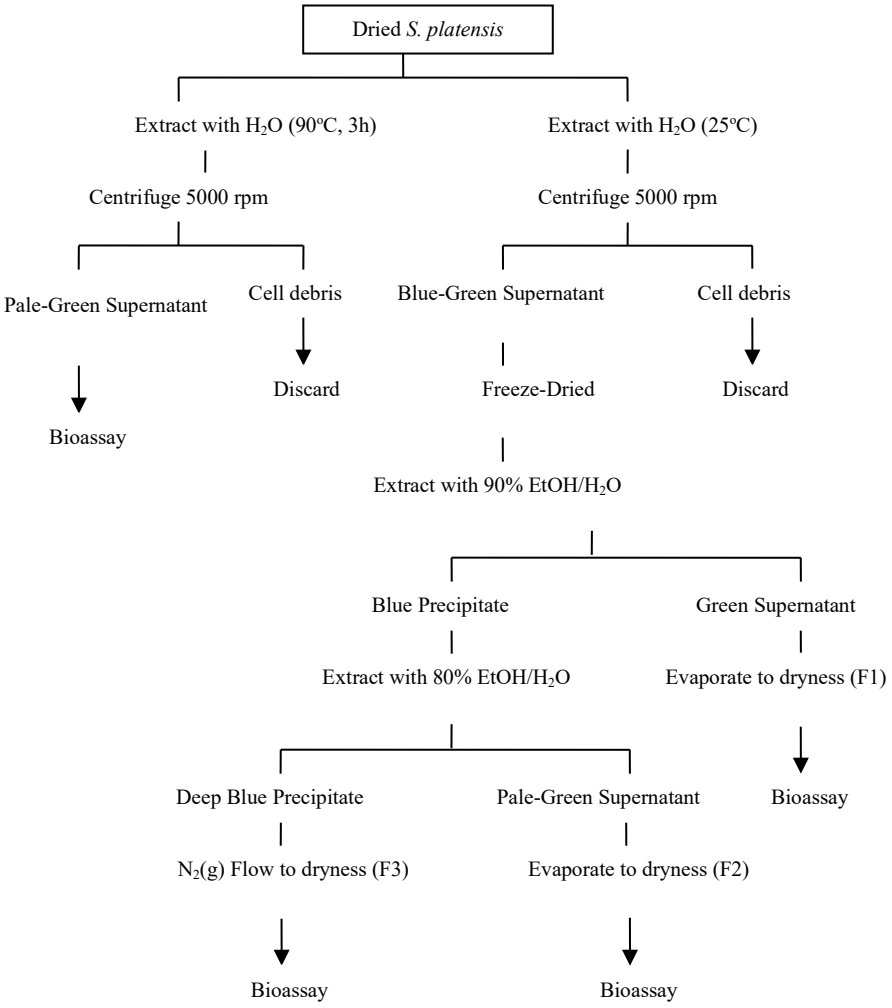

Figure 1. Flow chart of the extraction process of *S. platensis* to obtain the aqueous extract

2.3 Effect of subfractions of *S. platensis* aqueous extracts

Twenty milligrams of each subfraction were dissolved with distilled water in a 10 ml volumetric flask to make 2000 mg/l stock solution, and then each stock solution was measured for its UV-VIS absorption spectra and C-PC (mg/ml) was calculated as described below. The stock solutions were diluted to give final concentrations of 2000, 1000, 500, and 250 mg/l, respectively. Each subfraction was studied for its allelopathic activity. Five hundred microliters of each concentration were added to a glass vial (size 4.5 (height) x 2 (diameter) cm) containing germination paper, and then 10 seeds of the plant to be tested were placed on the germination paper. Controls received only distilled water (0.5 ml/vial). The treatments were carried out in quadruplicate in a completely randomized design. All vials were covered and placed at room temperature (32°C by day and 28°C by night) and under natural light conditions (6.00-18.00 h). After seven days, germination (%) and shoot and root length were recorded. Inhibition (%) relative to control, was calculated as:

Inhibition (% of control) = {100- [(*Sample extracts/Control*) x100]}

2.4 Effect of denatured C-phycocyanin

Ten milligrams of C-PC were dissolved with distilled water in a 10 ml volumetric flask and sealed with Parafilm. The solution was heated in a hot bath at 90°C for 3 h to obtain 1000 mg/l stock solution of denatured C-PC. The stock solution was allowed to cool to room temperature, the UV-VIS absorption spectra were measured and the concentration of C-PC (mg/ml) was calculated as described below. Afterwards, the denatured C-PC stock solution was diluted with distilled water to give the final concentrations of 1000, 500, 250, 125 and 62.5 mg/l, respectively. The denatured C-PC was evaluated for its allelopathic activity under similar conditions to those described above.

2.5 Preparation of *S. platensis* hot aqueous extract

The hot aqueous extract was prepared as an aqueous extract but in a hot bath with stirring at 90°C for 3 h. Afterwards, the mixture was centrifuged at 5000 rpm for 20 min, and the green supernatant (5% w/v stock solution) was diluted appropriately with distilled water to obtain the final concentrations of 5, 2.5, 1.25 and 0.625%, respectively. The remaining cell debris was discarded. The stock solution was diluted fifty-fold to prevent reabsorption effects. The purity of C-PC in the stock solution was determined from the UV-VIS absorption spectra and calculated as mg/ml.

2.6 Effect of hot aqueous extract

Five milliliters of each concentration of hot aqueous extract were added to a Petri dish (9.5 cm in diameter) containing germination paper followed by 20 seeds of tested plants. Growth occurred over seven days. Bioassay procedures and conditions were conducted under similar conditions as described above.

2.7 Spectroscopic measurements

All UV-VIS absorption spectra were recorded on a UV-VIS spectrometer (Thermo Electron, England) with a 1 cm path length. The ratio of A_{620}/A_{280} indicates the purity of C-PC, wherein A_{620} is the maximum absorbance of C-PC and A_{280} is the absorbance of total protein [15]. C-PC concentration in each solution was calculated as [16]:

Concentration of C-PC (mg/ml) = $[A_{620}-(0.474xA_{652})]/5.34$

2.8 Statistical analysis

Differences in the percentages of seed germination and root and shoot lengths were assessed by analysis of variance statistical methods. Comparisons between treatments were made at $p \leq 0.05$ probability level using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

The aqueous extract was dehydrated using a freeze-dryer to obtain a blue-green powder. One gram of the blue-green powder was fractionated by extraction with 90% (v/v) aqueous ethanol to obtain three subfractions, a green powder F1 (445.9 mg, 44.59%), a pale-green powder F2 (105 mg, 10.50%), and a deep-blue powder F3 (416.5 mg, 41.65%), respectively. Based on the UV-VIS spectra, the purity of C-PC for each subfraction was determined at A_{620}/A_{280} while a solution of commercial C-PC was used as the control. The results from Table 1 and Figure 2 indicated that all subfractions had a lower ratio than the aqueous extract. The deep-blue precipitate fraction (F3) showed an absorption band at 620 nm which represented the C-PC component. A high absorbance around 280 nm indicated that F3 had a higher protein content than did F2 and F1 [15, 17, 18]. As for the green powder (F1), a small absorbance band at 678 nm was found and it could be explained as a minor component of chlorophyll existed in the green powder [19].

As shown in Table 2, the results from the allelopathic assays indicated that subfractions F2 and F3 at concentration of 2000 mg/l were able to inhibit the germination of Chinese amaranth whereas subfraction F1 had no effect. For seedling growth, all subfractions at concentration of 2000 mg/l suppressed shoot length and at concentrations of 1000-2000 mg/l inhibited root length. The subfraction F3 had the highest inhibitory effect on the shoot and root growth of Chinese amaranth by 56.08 and 77.24%, respectively. In contrast, subfraction F2 at concentrations of 250-500 mg/l and subfraction F1 at concentration of 500 mg/l promoted root length. For barnyardgrass, none of the subfractions F1-F3 had any effect on seed germination. Subfractions F1 at concentration of 2000 mg/l, F2 at concentrations of 500-2000 mg/l, and F3 at concentrations of 250-2000 mg/l reduced shoot length of the plant. In particular, subfraction F3 at concentration of 2000 mg/l exhibited the highest inhibitory effect on shoot length by 52.82%. In terms of root growth, at concentration of 2000 mg/l, all subfractions inhibited root development. The highest effect was seen with subfraction F3 that inhibited root length by 69.65%. Subfractions F1 (at 250-1000 mg/l) and F2 (at 250-500 mg/l) promoted root length but other concentrations had no effect (Table 2). These results clearly indicated that the allelopathic effects of *S. platensis* extracts depended on its applied concentrations. At high concentrations, these extracts had a high inhibitory effect while at low concentration, they had a slight promotion effect [12].

Table 1. Absorbance, purity and concentrations of C-PC

Samples	A_{280}	A_{620}	Purity (A_{620}/A_{280})	C-PC (mg/ml)
Aqueous extract (5%) 1:50 dilution	0.118	0.138	1.169	0.020
Hot aqueous extract (5%) 1:50 dilution	0.165	0.063	0.381	0.007
F1 (2000 mg/l)	0.249	0.052	0.209	0.005
F2 (2000 mg/l)	0.266	0.086	0.323	0.009
F3 (2000 mg/l)	0.552	0.357	0.647	0.040
C-PC (1000 mg/l)	0.110	0.468	4.254	0.076
Denatured C-PC (1000 mg/l)	0.127	0.059	0.464	0.007

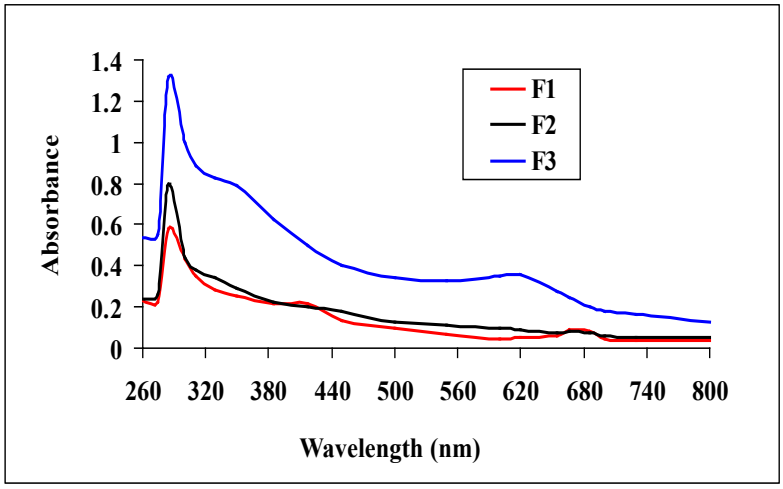

Figure 2. UV-VIS spectra of subfractions F1-F3

The results listed in Table 2 demonstrated that the deep-blue precipitate subfraction (F3), which also exhibited the highest constituency of C-PC, had the strongest allelopathic effect on tested plants. From experimental observations, it appeared that the color of the three subfractions was different mainly in paleness when compare to the aqueous extract. All subfractions of C-PC had purity ratio lower than that of the aqueous extract. It should be noted that C-PC might have become denatured during the extraction processes. This assumption would seem to be in agreement with the conclusions of Pasco *et al*. [20], who described the de-coloration of C-PC when it was dissolved in organic solvents such as aqueous ethanol. Other noted that aqueous extraction at temperature exceeding 40°C would undoubtedly cause C-PC to destabilize [19]. Furthermore, if the C-PC had completely denatured, it should have precipitated out of solution. The results from this experiment supported that the denatured component, decolorize C-PC, can play an allelopathic role.

The purity of the C-PC aqueous solution (pH = 7) was evaluated according to the purity ratio, A_{620}/A_{280}. The results indicated that after heating the C-PC solution at 90°C for 3 h the absorbance ratio was reduced from 4.254 to 0.464 (Table 1). The absorbance peak at 620 nm was lower and at 280 nm was higher (Figure 3), and the color had changed from deep blue to colorless. These findings suggested that the C-PC had become denatured under these conditions. In agreement with several previous reports by others, the effects of pH and temperature appeared to result in the degradation or denaturation of the aqueous C-PC extract [18-19, 21-23]. Our results indicated that when the temperature was high, denaturation increased. These results showed that for phycobiliprotein, denaturation and pH were inversely proportional. The denaturation of phycobiliprotein is known to be accompanied by a large loss of visible absorption caused by a change in the chromophores from a linear to a cyclic conformation. An extract that is gradually denaturing presents a visual color change from deep-blue to light-blue and finally to colorless, depending on the heating temperature and time length. Moreover, the absorption peak at 620 nm is known to decrease at the same time that increases occur at 280 nm and 370 nm increased [17-20, 23-25].

The results listed in Table 3 indicated that denatured C-PC had no effect on the seed germination of tested plants but exerted a high inhibitory activity on the growth of Chinese amaranth seedlings. It also showed a small inhibitory effect on branyardgrass growth. Denatured C-PC and

Table 2. Allelopathic effects of subfractions F1-F3 on seed germination and seedling growth of Chinese amaranth and barnyardgrass

Concentrations (mg/l)	% Inhibition on Chinese amaranth			% Inhibition on barnyardgrass		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
control	0^{c}	0^{d-f}	0^{f-h}	0^{a}	0^{h}	0^{c}
F1-250	-2.63^{c}	1.58^{d}	5.26^{ef}	0^{a}	-3.74^{i}	-16.25^{ef}
F1-500	2.63^{bc}	-6.98^{fg}	-12.54^{j}	0^{a}	-0.29^{h}	-18.08^{fg}
F1-1000	2.63^{bc}	2.03^{d}	16.56^{d}	0^{a}	-0.69^{h}	-9.95^{de}
F1-2000	7.90^{bc}	11.934^{c}	39.32^{c}	2.70^{a}	18.53^{e}	18.41^{b}
F2-250	0^{c}	-21.40^{h}	-6.50^{i}	0^{a}	-1.38^{hi}	-21.06^{fg}
F2-500	5.26^{bc}	-8.33^{g}	4.18^{e-g}	0^{a}	16.86^{e}	-24.71^{g}
F2-1000	2.63^{bc}	0.45^{de}	6.50^{e}	0^{a}	25.95^{d}	-2.65^{cd}
F2-2000	13.16^{b}	34.91^{b}	55.73^{b}	0^{a}	40.16^{b}	25.54^{b}
F3-250	0^{c}	-4.95^{d-g}	-3.41^{hi}	2.70^{a}	4.60^{g}	-5.97^{cd}
F3-500	0^{c}	-6.98^{fg}	-0.93^{gh}	0^{a}	8.17^{f}	0^{c}
F3-1000	2.63^{bc}	-5.86^{e-g}	18.42^{d}	2.70^{a}	31.82^{c}	-4.48^{cd}
F3-2000	50^{a}	56.08^{a}	77.24^{a}	8.11^{a}	52.82^{a}	69.65^{a}

Note: Mean values presented in each column followed by the same superscript letters indicate no significant different at $p \leq 0.05$.

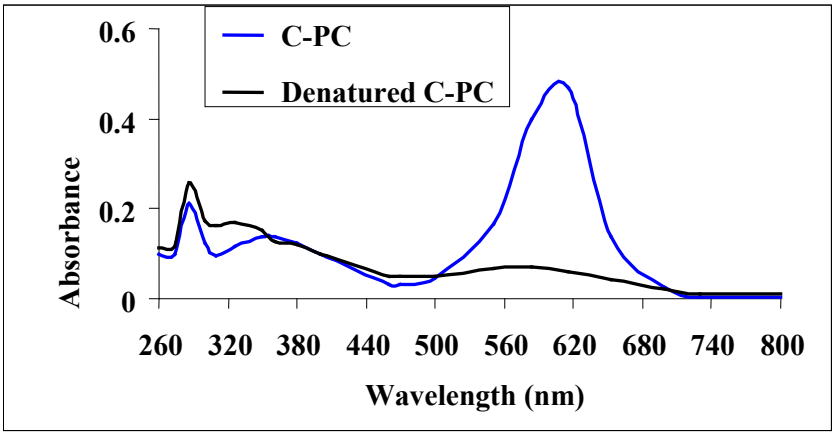

Figure 3. UV-VIS spectra of C-PC and denatured C-PC

Table 3. Allelopathic effects of denatured C-PC on seed germination and seedling growth of Chinese amaranth and barnyardgrass

Concentrations (ppm)	% Inhibition on Chinese amaranth			% Inhibition on barnyardgrass		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
control	0^{a}	0^{c}	0^{e}	0^{a}	0^{b}	0^{c}
62.5	0^{a}	0.75^{c}	2.29^{e}	0^{a}	-0.59^{b}	-2.33^{c}
125	0^{a}	1.50^{c}	32.80^{d}	-2.78^{a}	-1.18^{b}	-3.26^{c}
250	2.63^{a}	2.81^{c}	38.42^{c}	0^{a}	-1.45^{b}	-2.64^{c}
500	0^{a}	10.86^{b}	43.23^{b}	2.78^{a}	0.054^{b}	6.37^{b}
1000	2.63^{a}	26.40^{a}	75.69^{a}	2.78^{a}	10.75^{a}	27.02^{a}

Note: Mean values presented in each column followed by the same superscript letters indicate no significant different at $p \leq 0.05$.

C-PC [12] solutions exhibited inhibitory effects on the seedling growth of both Chinese amaranth and barnyardgrass. The denatured C-PC had an inhibitory effect on the shoot lengths of Chinese amaranth but the effect was not as robust as seen with the C-PC solution. Except at the concentration of 62.5 mg/l, all the applied concentrations of the denatured C-PC inhibited root length of Chinese amaranth to an extent that was similar to C-PC at concentrations of 125-1000 mg/l. These results indicated that denaturation of C-PC only slightly affected its allelopathic capabilities. In order to confirm this result, the allelopathic effect of hot aqueous extract from *S. platensis* was investigated.

The results listed in Table 4 and Figure 4 indicated that all the applied concentrations of hot aqueous extract from *S. platensis* were able to exert an allelopathic effect on seed germination and seedling growth of Chinese amaranth, and the extract was completely inhibitory at the concentration of 1.25%. Similar to the results obtained with denatured C-PC and C-PC, the hot aqueous extract exhibited a weakened inhibitory effect on the shoots of Chinese amaranth compared to aqueous extract [12].

Table 4. Allelopathic effects of hot aqueous extract on seed germination and seedling growth of Chinese amaranth and barnyardgrass

Concentrations (% w/v)	% Inhibition on Chinese amaranth			% Inhibition on barnyardgrass		
	Seed germination	Shoot length	Root length	Seed germination	Shoot length	Root length
control	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{d}
0.625	13.75^{b}	17.37^{b}	46.11^{b}	0^{c}	-6.83^{d}	10.79^{c}
1.25	100^{a}	100^{a}	100^{a}	23.19^{b}	20.89^{b}	53.31^{b}
2.5	100^{a}	100^{a}	100^{a}	97.10^{a}	96.61^{a}	100^{a}
5	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Note: Mean values presented in each column followed by the same superscript letters indicate no significant different at $p \leq 0.05$.

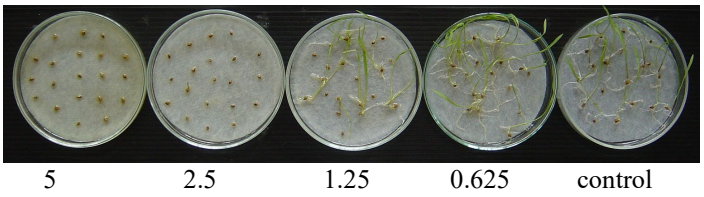

Figure 4. Allelopathic effects of hot aqueous extract on barnyardgrass

For barnyardgrass, the hot aqueous extract at concentration of 0.625% did not show an inhibitory effect on seed germination whereas the extract promoted shoot length and inhibited root length. Moreover, the hot extract at concentration of 1.25% inhibited seed germination and the growth of seedling and completely inhibited seed germination at concentration of 5%. The roots of tested plants were the most affected tissue and their growth was completely inhibited at concentration of 2.5%.

Hot aqueous extract had an A_{620}/A_{280} purity ratio of 0.38 and showed a very small absorbance band at 620 nm (Figure 5). This extract was then used to determine the concentration of C-PC and it was found that the concentration was reduced from 0.020 to 0.007 mg/ml (Table 1). It should be noted that denatured C-PC was verified as being present in this solution. This result was in agreement with previous studies found that the denatured C-PC can play an allelopathic role. These results are still doubtful though because the allelopathic effects of denatured C-PC may depend on its concentration in each extract. It is possible that the hot aqueous extraction may yield more crude extract than the aqueous extraction performed at room temperature. Here, our aqueous extract and hot aqueous extract were dehydrated to dryness and used to calculate the amount of crude extract in each solution. The results clearly showed that both solutions had very similar concentrations (107.20 mg/ 3 ml of 5% stock aqueous extract ~ 35,733.33 mg/l and 106 mg/3 ml of 5% stock hot aqueous extract ~ 35,333.33 mg/l).

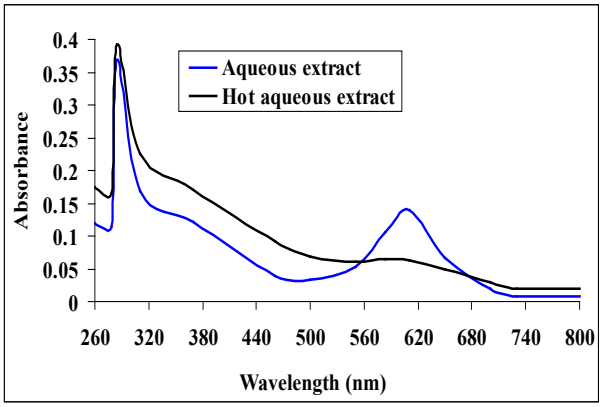

Figure 5. Absorption spectra of aqueous extract and hot aqueous extract

The finding in this experiment indicated that the denatured C-PC showed allelopathic activity on tested plants. These results were in agreement with several research studies previously performed to determine the allelopathic activity of degraded compounds. A good example is DIBOA

(2,4-dihydroxy-1,4-benzoxazin-3-one), an allelochemical with phototoxicity activity produced from many commercially important cultivates including wheat, maize and rice. The degradation of this compound in soil by fungi or bacteria yields a wide variety of compounds such as 2-bezoxazolinone (BOA), 2-aminophenol (APH), 2-amino-(3*H*)-phenoxazin-3-one (APO), and 2-acetylamino-(3*H*)-phenoxazin-3-one (AAPO). In particular, APO has shown excellent allelopathic effect when tested by others on plants [26-31]. In addition, 2-(2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one)-β-D-gluco pyranose (DIMBOA-Glc) is an allelochemical from wheat seedling which is stored in vacuoles. When this compound is leached from the roots to the soil, (DIMBOA-Glc) is transformed to its aglycone 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and then into 6-methoxy-benzoxazolin-2-one (MBOA). It has been reported that DIMBOA and MBOA are the dominant allelochemicals in wheat-weed allelopathic actions [32, 33]. Zhou *et al*. [23] investigated the antioxidant activity of C-PC and found that the ability of scavenging hydroxyl radicals greatly increased when this compound was denatured. They concluded that the phycobilin moiety was the main portion of C-PC involved in scavenging hydroxyl radicals. Taken together with our experimental results, it may be considered that denatured C-PC has a less robust allelopathic role; however, the exact mechanism by which the allelopathic activity of the denatured compound is meditated remains unknown.

4. Conclusions

In this study, the allelopathic activities of subfractions of aqueous extract, hot aqueous extract and denatured C-PC from *S. platensis* were evaluated against the dicotyledon, Chinese amaranth, and the monocotyledon, barnyardgrass. The results indicated that the inhibitory activity depended on the extract fraction and applied concentrations. Subfraction (F3), with the highest C-PC content, had the highest inhibitory effect on the tested plants compared to other fractions (F1 and F2). The inhibitory activity of the extracts increased when the applied concentrations increased. The hot aqueous extract exhibited the strongest overall inhibitory effect on the tested plant species. Denatured C-PC exerted a higher inhibitory effect on Chinese amaranth than it did on barnyardgrass. These effects were similar to that of the aqueous extract and C-PC.

5. Acknowledgements

The authors wish to thank the Research Foundation of King Mongkut's Institute of Technology Ladkrabang Thailand for providing financial support and the Department of Chemistry KMITL for making available laboratory facilities.

References

[1] Putnam, A.R. and Tang, C.S., 1986. Allelopathy: State of the science. In: A.R. Putnam and C.S. Tang, eds. *The Science of Allelopathy*. New York: Wiley-Interscience Publishers, pp. 1-19.
[2] Rice, E.L., 1984. *Allelopathy*. 2nd ed. New York: Academic Publishers.
[3] Kohli, R.K., Batish, D. and Singh, H.P., 1997. Allelopathy and its implication in agroecosystems. *Journal of Crop Production*, 1(1), 169-202.
[4] Singh, H.P., Batish D.R. and Kohli, R.K., 2001. Allelopathy in agroecosystems: an overview. *Journal of Crop Production*, 4(2), 1-41.

[5] Doan, N.T., Rickards, R.W., Rothschild, J.M. and Smith, G.D., 2000. Allelopathic actions of the alkaloid 12-*epi*-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix*. *Journal of Applied Phycol*ogy, 12(3), 409-416.

[6] Hirata, K., Yoshitomi, S., Dwi, S., Iwabe, O., Mahakhant, A., Polchai, J. and Miyamoto, K., 2003. Bioactivities of nostocine A produced by a freshwater cyanobacterium *Nostoc spongiaeforme* TISTR 8619. *Journal of Bioscience and Bioengineering*, 95(5), 512-517.

[7] Singh, D.P., Tyagi, M.B., Kumar, A., Thakur, J.K. and Kumar, A., 2001. Antialgal activity of a hepatotoxin-producing cyanobacterium, *Microcystis aeruginosa*. *World Journal of Microbiology and Biotechnology*, 17(1), 15-22.

[8] Jüttner, F., Todorova, A.K., Walch, N. and Philipsborn, W.V., 2001. Nostocyclamide M: a cyanobacterial cyclic peptide with allelopathic activity from Nostoc 31. *Phytochemistry*, 57(4), 613-619.

[9] Benedetti, S., Benvenuti, F., Pagliarani, S., Francogli, S., Scoglio, S. and Canestrari, F., 2004. Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae. Life Sciences,* 75(19), 2353-2362.

[10] Pardhasaradhi, B.V.V., Ali, A.M., Kumari, A.L., Reddanna, P. and Khar, A., 2003. Phycocyanin-mediated apoptosis in AK-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS. *Molecular Cancer Therapeutics*, 2(11), 1165-1170.

[11] Romay, C., Gonzalez, R., Ledon, N., Remirez, D. and Rimbau, V., 2003. C-Phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Current Protein and Peptide Science*, 4(3), 207-216.

[12] Charoenying, P., Chotsaeng, P. and Laosinwattana, C., 2010. Effects of *Spirulina platensis* and C-phycocyanin on seed germination and seedling growth of two monocot and dicot plants. *Allelopath Journal*, 25(2), 453-464.

[13] Zarrouk, C., 1966. Contribution á l'étude d'une cyanobacterie. *Influence de divers facteursphysiques et chimiques sur la croissancenet la photosynthése de Spirulina maxima* (Setch et Gardner) Geitler. Ph.D. University of Paris.

[14] Noguchi, Y., Ishii, A., Matsushima, A., Haishi, D., Yasumuro, K., Moriguchi, T., Wada, T., Kodera, Y., Hiroto, M., Nishimura, H., Sekine, M. and Inada, Y., 1999. Isolation of biopterin-α-glucoside from *Spirulina (Arthrospira) platensis* and its physiologic function. *Marine Biotechnology*, 1(2), 207-210.

[15] Rito-Palomares, M., Nunez, L. and Amador, D., 2001. Practical application of aqueous two-phase systems for the development of a prototype process for C-phycocyanin recovery from *Spirulina maxima*. *Journal of Chemical Technology and Biotechnology*, 76(12), 1273-1280.

[16] Bennett, A. and Bogorad, L., 1973. Complementary chromatic adaptation in a filamentous blue-green algae. *The Journal of Cell Biology*, 58(2), 419-435.

[17] MacColl, R. and Guard-Friar, D., 1987. *Phycobiliproteins*. Boca Raton: CRC Press.

[18] MacColl, R., Kapoor, S., Montellese, D.R., Kukadia, S. and Eisele, L.E., 1996. Bilin chromophores as reporters of unique protein conformations of phycocyanin 645. *Biochemistry,* 35(48), 15436-15439.

[19] Sarada, R., Pillai, M.G. and Ravishankar, G.A., 1999. Phycocyanin from *Spirulina* sp. influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochemistry*, 34(8), 795-801.

[20] Pasco, D.S., Pugh, N.D., Elsohly, M., Ross, S. and Elsohly, N.M., 2007. *Potent Immunostimulants from Microalgae*. U.S. Pat. 7,205,284 B2.

[21] Edwards, M.R., MacColl, R. and Eisele, L.E., 1996. Some physical properties of an unusual C-phycocyanin isolated from a photosynthetic thermophile. *Biochimica et Biophyssica Acta (BBA)-Bioenergetics*, 1276(1), 64-70.

[22] Antelo, F.S., Costa, J.A.V. and Kalil, S.J., 2008. Thermal degradation kinetics of the

phycocyanin from *Spirulina platensis*. *Biochemical Engineering Journal*, 41(1), 43-47.

[23] Zhou, Z-P., Liu, L-N., Chen, X-L., Wang, J-X., Chen, M., Zhang, Y-Z. and Zhou, B-C., 2005. Factors that effect antioxidant activity of C-phycocyanins from *Spirulina platensis*. *Journal of Food Biochemistry*, 29(3), 313-322.

[24] Han, B-H., Pyo, M-K. and Yang, S.J., 2003. *Denatured Spirulina and Manufacturing Method Thereof*. International Patent, WO/2003/080811.

[25] O'Carra, P., Murphy, R.F. and Killilea, S.D., 1980. The native forms of the phycobilin chromophores of algal biliproteins. A clarification. *The Biochemical Journal*, 187(2), 303-309.

[26] Macias, F.A., Marin, D., Oliveros-Bastidas, A., Varela, R.M., Simonent, A.M., Carrera, C. and Molinillo, J.M., 2003. Allelopathy as a new strategy for sustainable ecosystems development. *Biological Science in Space*, 17(1), 18-23.

[27] Macias, F.A., Oliveros-Bastidas, A., Marin, D., Castellano, D., Simonet, A.M., Simonent, A.M. and Molinillo, J.M., 2005. Degradation studies on benzoxazinoids. Soil degradation dynamics of (2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *Journal of Agricultural and Food Chemistry*, 53(3), 554-561.

[28] Gagliardo, R.W. and Chilton, W.S., 1992. Soil transformation of 2(2H)-benzoxazolone of rye into phototoxic 2-amino-3H-phenoxazin-3-one. *Journal of Chemical Ecology*, 18(10), 1683-1691.

[29] Niemeyer, H.M., 1988. Hydroxamic acids (4-Hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the gramineae. *Phytochemistry*, 27(11), 3349-3358.

[30] Yue, Q., Bacon, C.W. and Richardson, M.D., 1998. Biotransformation of 2-benzoxazolinone and 6-methoxy-benzoxazolinone by *Fusarium moniliforme*. *Phytochem*istry, 48(3), 451-454.

[31] Zikmund Ova, M., Dandrarov, K., Hesse, M. and Werner, C., 2002. Hydroxylated 2-amino-3H-phenoxazin-3-one derivatives as products of 2-hydroxy-l,4-benzoxazin-3-one (HBOA) biotransformation by *Chaetosphaeria* sp., an endophytic fungus from *Aphelandra tetragona*. *Zeitschrift für Naturforschung*, 57c(7-8), 660-665.

[32] Macias, F.A., Oliveros-Bastidas, A., Martin, D., Castellano, D., Simonet, A.M. and Molinillo, J.M.G, 2004. Degradation studies on benzoxazinoids. Soil degradation dymanics of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. *Journal of Agricultural and Food Chemistry*, 52(21), 6402-6413.

[33] Understrup, A.G., Ravnskov, S., Hansen, H.C.B. and Fomsgaard, I.S., 2005. Biotransformation of 2-benzoxaziniods to 2-amino-(3H)-phenoxazin-3-one and 2-acetylamino-(3H)-phenoxazine-3-one in soil. *Journal of Chemical Ecology*, 31(5), 1205-1222.