

ANTIBACTERIAL ACTIVITY OF EXTRACTS DERIVED FROM THE UNICELLULAR GREEN ALGAE, *CHLORELLA* spp.

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ABSTRACT

Antibacterial substances from five strains of unicellular green algae, *Chlorella* spp., were extracted in water : methanol : chloroform (1 : 2 : 1, v/v). The highest yield of crude algal extract (0.24 g/g dry weight) was obtained from *Chlorella* spp. A. 0505. Various concentrations of the extracts were tested against 6 genera of bacteria for antibacterial activity by the disc diffusion method. It was found that the crude algal extracts (4 mg/disc) from *Chlorella vulgaris* TISTR 8580 strongly inhibited the growth of *Staphylococcus aureus* and *Streptococcus pyogenes*. Moreover, the extracts from *Chlorella* spp. A. 0505 and *Chlorella vulgaris* TISTR 8261 have been found to effectively inhibit the growth of *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively.

KEYWORDS: Antibacterial activity, unicellular green algae, *Chlorella* spp.

1. INTRODUCTION

The present of bioactive natural products in extracts of algae is well known. Several screening surveys have revealed that many algae possess antibacterial [1, 2, 3] antifungal [2, 3, 4] antiviral [5] antialgal [6] and anticoagulant [8] activities.

Chlorella Beji (Family Chlorellaceae, Order Chlorellales) is a unicellular green alga common in freshwater bodies. Pratt *et al.* [9] demonstrated that chlorellin an antibacterial substance from *Chlorella* was active against five bacterial species. Since then, many bioactive compounds have been isolated from various algae including fatty acids, terpenes, bromophenols, halogenated compounds, peptides, polyphenols, and polysaccharides [1, 10, 11, 12, 8].

In this study, we examined the extracts of the unicellular green algae, *Chlorella* for antibacterial activity.

2. MATERIALS AND METHODS

Microorganism

The Chlorophyte *Chlorella* spp. A.0505, *Chlorella* spp. D.1708 and *Chlorella* spp. E.1708 were isolated from natural freshwater ponds. *Chlorella vulgaris* TISTR 8261 and *C. vulgaris* TISTR 8580 obtained from the Microbiology Resource Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand.

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Cultural condition

Algae cultures were cultivated in 300 mL glass columns each containing 200 mL of modified N-8 medium [13]. Cultures were stirred by bubbling air at 30 °C under continuous illumination with cool – white fluorescent lamps, (light intensity of 2,400 lux) for 10 days. The algal cells were harvested by centrifugation at 3,600 rpm for 20 min at 4 °C, washed twice with distilled water following which distilled water was added to the pellets (1 : 1, w/v).

Preparation of crude extracts

Crude extracts were prepared from five algal cultures. Each algal culture was sonicated on ice by sonic vibra cell. The supernatant was extracted with water : méthanol : chloroform (1 : 2 : 1, v/v), The extracted solvent was pooled and dried by a rotary evaporator.

Antibacterial assay

Antibacterial assays were conducted on six species of bacteria using the disc diffusion method. [2] Bacteria used for assay were *Bacillus subtilis* *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* spp. This was determined by observing the inhibition of their growth at 37 °C on nutrient agar plate for 24 h. Algae crude extracts (1, 2, 3 and 4 mg) were transferred to 6 mm paper discs, which were then applied to plates seeded with the respective test strains. Inhibition results were expressed as the width of the clear halo surrounding each disc after 24 h at 37 °C.

Statistic

The statistical significance of the data was determined by Least significant different test (LSD). All *P* values less than 0.01 were considered significant.

3. RESULTS AND DISCUSSION

Crude extract production

The crude extract contents of the five *Chlorella* strains are shown in Table 1. The highest yield of crude extract was 0.24 g/g dry weight, which was obtained from *Chlorella* spp. A.0505.

Table 1 The crude extract production from 5 strains of *Chlorella* spp.

Strain	Crude extract content g/g (dry wt)
<i>Chlorella</i> spp. A.0505	0.24
<i>Chlorella</i> spp. D.1708	0.11
<i>Chlorella</i> spp. E.1708	0.21
<i>Chlorella vulgaris</i> TISTR 8261	0.06
<i>Chlorella vulgaris</i> TISTR 8580	0.12

Antibacterial activity

The crude extracts of three isolated strains and two strains of stock cultures were tested against six bacterial strains. All extracts showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* (Table 2) but exhibited no activity on *Escherichia coli* and *Salmonella* spp. The extracts of *C. vulgaris* TISTR 8261 and *C. vulgaris* TISTR 8580 were most active against *S. aureus* and *Strep. pyogenes* (*P* < 0.01). The extract of *C. vulgaris* TISTR 8580 produced the largest inhibition zone (diameter of 29.1 mm) at the concentration of 4 mg/disc, indicating that *Strep. pyogenes* was the most susceptible to the inhibitory effects of the *Chlorella* extracts tested.

Table 2 Comparision of antibacterial activities of crude extracts (4 mg/disc) from 5 different strains of *Chlorella* spp.

Strain	Inhibition zone (mm)					
	Gram - positive			Gram - negative		
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella</i> spp.
<i>Chlorella</i> spp. A.0505	-	7.6 c	23.5 c	-	18.4 a	-
<i>Chlorella</i> spp. D.1708	-	8.0 c	20.0 d	-	11.2 b	-
<i>Chlorella</i> spp. E.1708	-	10.08 b	27.4 b	-	9.7 c	-
<i>C. vulgaris</i> TISTR 8261	8.1	17.4 a	28.4 a	-	-	-
<i>C. vulgaris</i> TISTR 8580	-	17.7 a	29.1 a	-	-	-

- no activity

Means followed by the same letter were not significantly different ($P > 0.01$, one-way ANOVA; LSD test)

Chang *et al.* [1] demonstrated that, a crude extract of *Dunaliella primolelecta* strongly inhibited the growth of *S. aureus*, but exhibited no activity towards *E. coli* and *Sal. typhimurium*. Pushparaj *et al.* [3] found that cyanobacterium *Nodularia harveyana* exhibited antibacterial activity against Gram-positive but not Gram-negative bacteria. Similarly, Vlachos *et al.* [2] showed that the extracts of southern African seaweeds were more active against Gram-positive than Gram-negative bacteria.

In this study, *B. subtilis* was inhibited only by the extract of *C. vulgaris* TISTR 8261 at the concentrations of 2, 3 and 4 mg/disc, but exhibited no activity at the concentration of 1 mg/disc (Table 3). This result was similar to those reported by Pratt *et al.* [9]. It was found that *Strep. pyogenes* was more sensitive than *B. subtilis* whereas Vlachos *et al.* [2] reported that *B. subtilis* was the most susceptible organism.

Table 3 Effect of crude extract concentrations on antibacterial activity in *Chlorella* spp.

Strain/concentration (mg/disc)	Inhibition zone (mm)					
	Gram - positive			Gram - negative		
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella</i> spp.
<i>Chlorella</i> spp. A.0505						
1	-	6.2	19.5	-	6.7	-
2	-	6.6	20.9	-	9.7	-
3	-	7.0	22.0	-	16.5	-
4	-	7.6	23.5	-	18.4	-
<i>Chlorella</i> spp. D.1708						
1	-	-	13.8	-	7.4	-
2	-	-	15.2	-	8.6	-
3	-	6.4	16.2	-	9.7	-
4	-	8.0	20.0	-	11.2	-
<i>Chlorella</i> spp. E. 1708						
1	-	8.5	22.0	-	7.4	-
2	-	9.1	24.3	-	8.2	-
3	-	10.4	26.0	-	8.8	-
4	-	10.8	27.4	-	9.7	-

- no activity

Table 3 Effect of crude extract concentrations on antibacterial activity in *Chlorella* spp.
(contd.)

Strain/concentration (mg/disc)	Inhibition zone (mm)					
	Gram - positive			Gram - negative		
	<i>Bacillus</i> <i>subtilis</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Streptococcus</i> <i>pyogenes</i>	<i>Escherichia</i> <i>coli</i>	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Salmonella</i> <i>spp.</i>
<i>C. vulgaris</i> TISTR 8261						
1	-	13.8	23.6	-	-	-
2	6.4	15.0	25.8	-	-	-
3	7.3	16.0	26.4	-	-	-
4	8.1	17.4	28.4	-	-	-
<i>C. vulgaris</i> TISTR 8580						
1	-	15.1	24.7	-	-	-
2	-	15.9	25.7	-	-	-
3	-	16.4	27.0	-	-	-
4	-	17.0	29.1	-	-	-

Pseudomonas aeruginosa was inhibited by *Chlorella* spp. A.0505, *Chlorella* spp. D.1708 and *Chlorella* spp. E.1708. The extracts at all concentrations (1, 2, 3 and 4 mg/disc) inhibited the growth of *Pseudomonas aeruginosa* and the inhibition zones were increased with increase in extract concentration (Table 3). Robles *et al.* [14] suggested that antibiosis in algae was extremely complex and involved numerous different activities which manifested themselves differently with respect to habitat, season and life history stage.

REFERENCES

- [1] Chang, T., S. Ohta, N. Ikegami, H. Miyata, T. Kashimoto and M. Konko. Antibiotic substances produced by a marine green alga, *Dunaliella primolecta*. *Biores. Technol.* 44, 1993, 149 – 153.
- [2] Vlachos, V., AT. Critchley and AV. Holy. Antimicrobial activity of extracts from selected southern African marine macroalgae. *South African J. of science.* 93, 1997, 328 – 332.
- [3] Pushparaj, B., E. Pelosi and F. Juttner. Toxicological analysis of the marine cyanobacterium *Nodularia harveyana*. *J. appl. Phycol.* 10, 1999, 527 – 530.
- [4] Anggadiredja, J., LB. Astuti, W. Sujatmiko and H. Purwoto. Antifungal activity of *Laurencia* sp. : a preliminary study. In : *Proceedings of the 2nd Asia – Pacific marine biotechnology conference and 3rd Asia-Pacific conference on algal biotechnology*. 7 – 10 May 1997 Phuket Thailand. 1997, 249 – 254.
- [5] Patterson, GML., KK. Baker, CL. Baldwin, CM. Bolis, FR. Capland, LK. Larsen, IA. Levine, RE. Moore, CS. Nelson, KD. Tschappat, GD. Tuang, MR. Boyd, JH. Cardellina, RP. Collins, KR. Gustafson, KM. Snader, OS. Weislow and RA. Lewin. 1993 Antiviral activity of cultured blue-green algae (Cyanophyta). *J. Phycol.* 29 : 125 – 130.
- [6] Schlegel, I., NT. Done, ND. Chazal and GD. Smith. Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. appl. Phycol.* 10, 1999, 417 – 479.
- [7] Matsubara, k., Y. Matsuura, K. Hori and K. Miyazawa. An anticoagulant proteoglycan from the marine green alga, *Codium pugniformis*. *J. appl. Phycol.* 12, 2000, 9 – 14.
- [8] Pratt, R., TC. Daniels, JJ. Eiler, JB. Gunnison, WD. Kumler, JF. Oneto and LA. Strait. Chlorellin, an antibacterial substance from *Chlorella*. *Science.* 99, 1994, 351 – 352.

- [9] Shamsudin, L. Useful Biochemical components of selected malaysian brown seaweeds. In : *Proceedings of the 2nd Asia – Pacific marine biotechnology conference and 3rd Asia-Pacific conference on algal biotechnology. 7 – 10 May 1997 Phuket Thailand.* 1997, 235 – 242.
- [10] Qingxiang, L., Y. Xiaojun, F. Xiao and H. Lijun. Studies on extraction procedure of phlorotannins. In : *Proceedings of the 2nd Asia – Pacific marine biotechnology conference and 3rd Asia-Pacific conference on algal biotechnology. 7 – 10 May 1997 Phuket. Thailand.* 1997, 231 – 233.
- [11] Kodani, S., K. Ishida and M. Murakami. Dehydroradiosumin, a trypsin inhibitor from the cyanobacterium *Anabaena cylindrica* *J. Nat. Prod.* 61, 1998, 6(854 – 856).
- [12] Attasampunna, P. TISTR culture collection fifth edition Bangkok MIRCEN. Thailand Institute of Scientific and Technological Research Bangkok, Thailand, 1995.
- [13] Robles Centeno, PO., DL. Ballantine and WH. Gerwick. Dyanmics of antibacterial activity in three species of Caribbean marine algae as a function of habitat and life history. In : Robles Centeno, PO. and DL. Ballantine. 1999. Effects of culture conditions on production of antibiotically active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). I. Light. *J. appl. Phycol.* 10, 1996, 453 – 460.