

# 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 : methanol : 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* sp. 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** Comparison 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 primolecta* 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<br>(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>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.

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