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# EFFECTS OF ENZYME AND MICROWAVE ON LECTIN EXTRACTION FROM *Chaetomorpha aerea* ALGA

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**ABSTRACT**

Plant-based lectins are promising biotechnological sources that also gain prominence when applied to pharmacology, especially seaweed lectins. Lectins exhibit various biological activities such as antioxidant, antifungal, antibacterial, and anticancer. *Chaetomorpha aerea* contains lots of bioactive compounds, notably lectin. Viscozyme L enzyme and microwave were used to improve the efficiency of lectin extraction from *C. aerea* alga. This study investigated the effects of Viscozyme L and microwave treatment on lectin extraction from *C. aerea*. The results showed that the enzyme-assisted extraction (EAE) method resulted in a protein content of 0.098 g/g DM, and hemagglutinating activity (HA) of 3,521.075 HU/g. During microwave-assisted extraction (MAE), the obtained protein content was 0.101 g/g DM and the HA was 4,377.739 HU/g.

*Keywords: Chaetomorpha aerea*, extraction, lectin, microwave, Viscozyme L.

## 1. INTRODUCTION

Lectins are a diverse class of proteins found in all kingdoms of life, especially plants. Plant lectins are carbohydrate-binding proteins containing at least one particular lectin domain, which enables them to recognize and bind carbohydrate structures specifically [1, 2]. Lectins have many valuable biological activities such as antioxidant, antifungal, antibacterial, anti-inflammatory, antivirus [3]. Thus, lectins are currently a subject of intensive studies and are considered important in medicine and technology. Lectins have been isolated from various algae. Lectin extracted from *Eucheuma serra* induces cell death in human cervical cancer cells [4]. Lectins isolated from *Microcystis viridis* and *Scytonema varium* also inhibit viral infection and prevent the entry of Hepatitis C into human hepatocytes [5].

*C. aerea* is common in shrimp ponds in the Mekong Delta, Vietnam. This alga is a brackish water alga belonging to the genus *Chaetomorpha*. The excessive proliferation of *C. aerea* prevents the proper development of the phytoplankton portion that shrimps need to develop [6]. Moreover, when this alga dies, the bacteria that decompose the dead algae will suck up oxygen from the water, so the oxygen in the water is consumed, and it is impossible for aquatic life to survive. In recent years, several studies have investigated the potential positive exploitation of these algae. There are various biologically active substances such as galactan sulfate, polysaccharides, and allophycocyanin... have been found in *C. aerea* [6, 7]. In addition, the alga contains remarkable protein content (13.1 - 16.4% dry mass), so there is a lot of potential to exploit lectins [8]. Thus, it is essential to exploit valuable components like lectin from *C. aerea* for further practical applications to enhance the value of this material and reduce the negative environmental effects of its decomposition.

This study aimed to determine the effects of extraction conditions for lectin from *C. aerea* alga with the assistance of the enzyme Viscozyme L and microwave treatments. In the EAE method, the enzyme concentration, time, and temperature were studied, whereas the material/solvent ratio, microwave power and time were also studied in the MAE method. These findings provide solid information on lectin extraction for further studies and the application of these compounds to functional food and pharmaceutical purposes.

## 2. MATERIALS AND METHODS

### 2.1. Materials

*C. aerea* alga was collected from abandoned ponds in Gia Thuan commune, Go Cong Dong district, Tien Giang province (Vietnam) in mid-May 2023 and transported within the same day to the laboratory. The morphology and structure of the collected algae samples were compared with the identification of *C. aerea* alga, as documented by Huang [9] and Titlyanov [10]. *C. aerea* was rinsed with distilled water, cleaned of impurities (gravel, sand, leaves, snails, etc.), dried until moisture content < 10%, ground to a fine powder, and sifted through an 80 mesh sieve. After that, the fine powder was stored in the zipper storage bag at −20 °C.

Viscozyme L (100 FBGU/g) was obtained from Novozymes (Bagsvaerd, Denmark). BSA (Bovine Serum Albumin) and Folin–Ciocalteu reagent by Ciocalteu (Merck, Germany) were used. Other reagents of the highest purity were obtained from local suppliers.

UV-VIS spectrophotometer V-730 (Kern - Germany), cold centrifuge Hermle, Z 216 MK (Germany), thermostatic tank Memmert WNB22, 22 L (Germany) were used.

### 2.2. Methods

#### *2.2.1. Effects of Viscozyme L on the lectin extraction*

The mixture included 1 g biomass sample (based on dry mass) and phosphate buffer (0.1 M, pH 7) at the ratio of 1/20 (w/v). Viscozyme L was added to the mixture with the investigated concentrations (0, 0.2, 0.4, 0.6, 0.8, 1% DM) and extracted at 40 °C for 1 h. Then, the temperature (30, 40, 50, 60 °C) was studied with the enzyme concentration from the previous experiment and extracted for 1 h. Finally, the extraction time (1, 2, 3, 4 h) was studied with the selected enzyme concentration and temperature. The mixture was centrifuged at 5,000 rpm for 30 minutes to collect the supernatant and determine the total protein content and hemagglutination activity.

#### *2.2.2. Effects of microwave on the lectin extraction*

The mixture included 1 g of biomass sample (based on DM) and phosphate buffer (0.1 M, pH 7) at the investigated ratios (1/20, 1/25, 1/30, 1/35, 1/40 w/v). The extraction was performed at a microwave power of 180 W for 16 seconds. Next, the microwave power (90, 180, 270, 360, 450 W) was studied with the solvent/material ratio from the results of the previous experiment for 16 seconds. Finally, the microwave time (8, 16, 24, 32, 40 seconds) was studied with the previously chosen conditions. The mixture was centrifuged at 5,000 rpm for 30 minutes to collect the supernatant and determine the total protein content and hemagglutination activity.

#### *2.2.3. Analysis methods*

The protein concentration was determined by using Lowry's method, using BSA as a standard [11]. BSA solutions in a series of concentrations (20, 40, 60, 80, and 100 µg/mL) were used as standards. Standards and samples with 0.4 mL each were pipetted on test tubes, and 0.25 mL of Folin & Ciocalteu's phenol reagent was added. After 30 minutes of incubation

at ambient temperature and protection from light, the samples were measured with a UV detector at a wavelength of 750 nm using a UV-VIS spectrophotometer V-730.

Hemagglutination is a process in which blood cells are cross-linked via multivalent molecules. The hemagglutination assay is a method to obtain semi-quantitative data on the sugar-binding and specificity of a lectin [1]. The HA was carried out by measuring the maximally visible agglutination on 2% suspension of rabbit red blood cells, and the hemagglutination activity (HU/mg) was expressed as the number of hemagglutination per mg protein [12, 13]. In the assay for HA, a serial two-fold dilution of the lectin solution in microtiter U-plates (50 mL) was mixed with 50 mL of a 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20 °C. The results were read after about 1 h when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg of protein [12, 13].

$$\mathrm{HA} = \frac{\mathrm{V} \times 2^{\mathrm{n}}}{\mathrm{protein_{total}}} \quad (Eq.\ 1)$$

where V is the total volume (mL), n is the number of dilutions, and $\mathrm{protein_{total}}$ is the total protein content.

*2.2.4. Data analysis*

All experiments were arranged in 3 replicates, and the results are presented as mean ± SD. The arrangement and treatment were supported by statistical software Minitab 20 and analysis of variance (ANOVA) ($p < 0.05$) to evaluate the difference between treatments. The chart is drawn using Microsoft Excel 2019 software.

## 3. RESULTS AND DISCUSSION

### 3.1. Effects of enzyme concentration, temperature, and time on lectin extraction

The effects of three independent variables (enzyme concentration, extraction temperature, and extraction time) on the protein content and hemagglutinating activity have been studied. The effects of these factors are shown in Fig. 1.

The protein content increased from 0 to 1% enzyme concentration, but the HA decreased from 0.4 to 1% (Fig. 1A). Viscozyme L disrupts the *C. aerea* cell wall's structural integrity, thereby enhancing bioactive extraction from this alga [14]. Viscozyme L hydrolyzes the cell wall and increases its permeability, leading to increased extraction yield. Depending on the enzyme concentration and substrate concentration, the reaction rate of the degradation process takes place quickly or slowly. When the enzyme concentration increased to 0.4%, the reaction rate increased. The protein content reached 0.093 ± 0.003 g/g DM, and the HA also was 3430.266 ± 308.000 HU/g. However, as the enzyme concentration was further increased, the effect on the reaction rate began to decline until a stage was reached where increasing the substrate concentration had little effect on the reaction rate. In addition, when the enzyme concentration is greater than 0.4%, non-protein compounds were also released into the solvent, reducing the HA of lectin. Thus, 0.4% enzyme concentration was chosen for the following investigation.

The protein content and the HA increased from 30 to 50 °C, but both decreased from 50 to 60 °C (Fig. 1B). Viscozyme L works optimally at temperatures of 50 - 55 °C [15]. For this reason, relatively higher biomass concentrations can be hydrolyzed at higher temperatures. When the extraction temperature is lower or higher than the optimal temperature, the enzyme activity and the reaction rate decrease. Increasing temperature causes the kinetic energy of enzymes and substrates to increase. Reactions occur faster, and protein separation efficiency increases. At 50 °C, both the protein content and the HA contents reached maximum values. The protein content reached 0.097 ± 0.004 g/g DM, and the HA also was 3469.082 ± 86.921

HU/g. However, high temperatures might result in enzyme denaturation. At lower temperatures than the optimal temperature, the substrate and enzyme molecules move slowly. The low frequency of collisions between them leads to less formation of enzyme-substrate complexes and a reduced reaction rate.

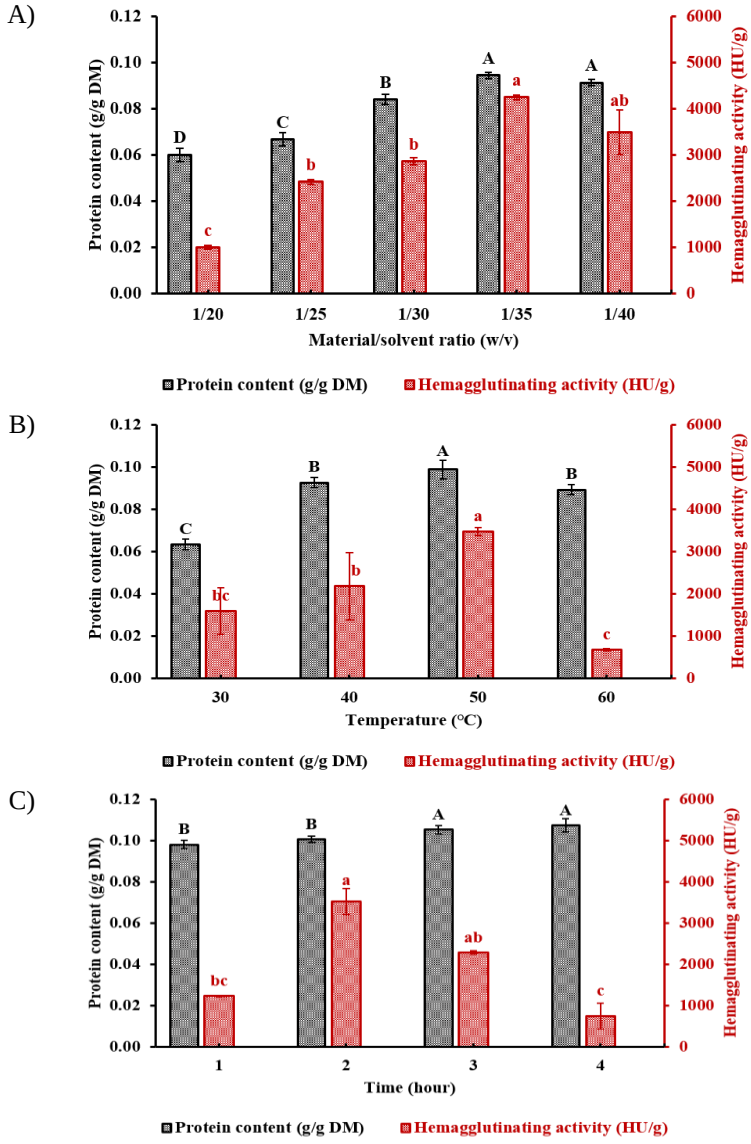


*Fig. 1.* Effects of enzyme concentration (A), temperature (B), and extraction time (C) on protein content and HA of lectin

*Note: Different letters in the column indicate a statistically significant difference at $p < 0.05$*

The protein content increased gradually with extraction time, but the HA decreased gradually when the extraction time was longer than 2 hours (Fig. 1C). When the catalytic time was too long, proteins and lectins were decomposed to a limited extent. Protein content increased insignificantly, but the HA decreased sharply because many compounds with properties different from lectin were released into the solvent. Hence, the optimal time for lectin extraction from *C. aerea* is 2 hours. The protein content reached 0.098 ± 0.001 g/g DM and the HA also was 3521.075 ± 317.692 HU/g.

The protein recovery was 0.098 g/g DM with an efficiency of 51%, similar to the results of the study on protein extraction from red seaweed *Palmaria palmata* with an enzyme concentration of 0.4% and a recovery efficiency of 45% [16]. The results were also similar to the results of the study on protein extraction from *Phaseolus vulgaris* at 50 °C [17] and the time extraction for 110 min with *Chaetomorpha* sp. [8].

### 3.2. Effects of the material/solvent ratio, microwave power, and time treatment on lectin extraction

The effects of three independent variables (the material/solvent ratio, microwave power, and extraction time) on the protein content and hemagglutinating activity have been studied. The effects of these factors are shown in Fig. 2.

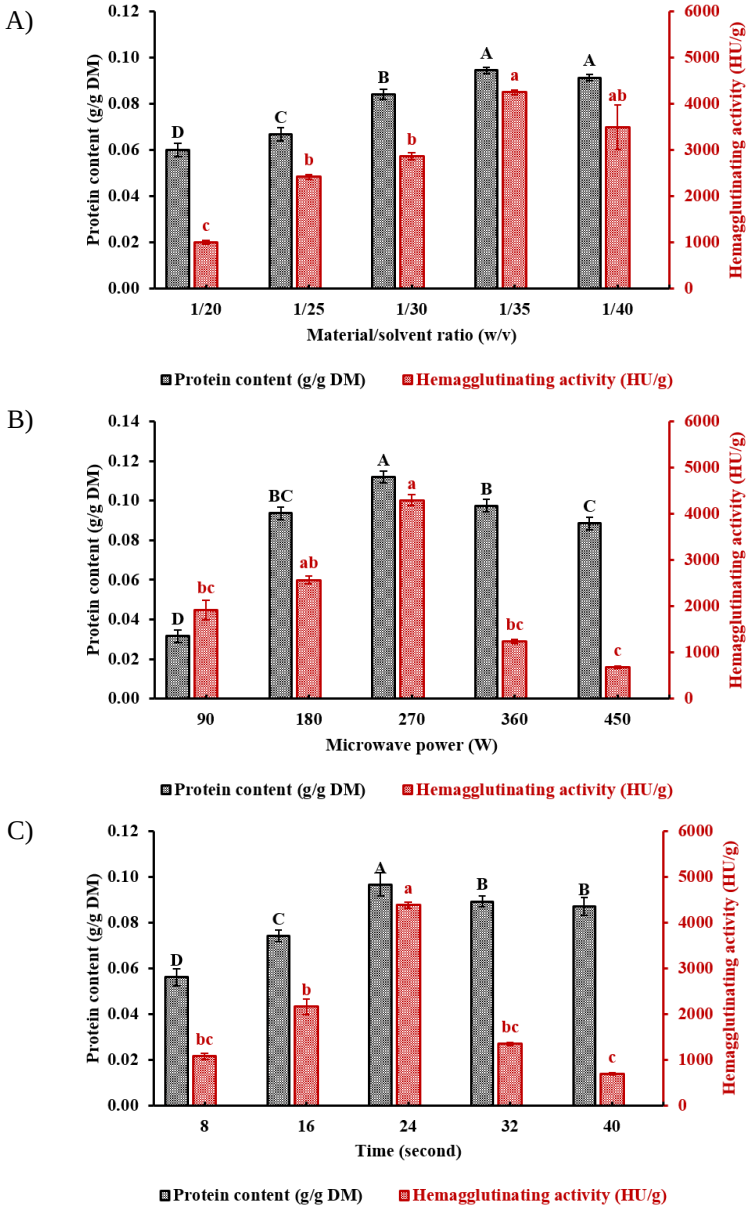


*Fig. 2.* Effects of material/solvent ratio (A), microwave intensity (B), and extraction time (C) on protein content and HA of lectin

*Note: Different letters in the column represent a statistically significant difference at $p < 0.05$*

From Fig. 2, suitable microwave treatment conditions to extract lectin from *C. aerea* are material/solvent ratio 1/35 (w/v), microwave power 270 W for 24 seconds. The protein content reached 0.101 ± 0.005 g/g DM, and the HA also was 4377.739 ± 68.551 HU/g. When increasing the solvent volume (from 1/20 to 1/35 w/v), more solutes in the cell will move out of the cell to achieve equilibrium inside and outside the cell. Once there is a certain amount of solvent and the cell material has fully diffused into it. The protein content obtained remains constant because a material equilibrium has been achieved. [18]. In addition, increasing the amount of extraction solvent will simultaneously separate other proteins with the same absorption wavelength as lectin from the cytoplasm, reducing the HA. Microwave power is an important factor affecting the efficiency of microwave-assisted extraction. The protein content and HA increased continuously and reached the highest value at 270 W. When the microwave power was higher than 270 W, the protein content and HA value decreased. Increasing the microwave power can strengthen the molecular interaction between the electromagnetic field and the sample and improve the extraction efficiency [19]. However, the continued increase in microwave power may cause degradation of target antioxidants [20]. Thus, a microwave power of 270 W was used to extract lectin. The extraction time of 24 seconds was the highest efficiency. As the extraction time exceeded 24 seconds, the protein content decreased slightly, and the HA significantly decreased. When the extraction time was less than 24 seconds, the microwave action time was short and the extraction might be insufficient. However, prolonged high temperatures due to too long extraction time can increase the ability to extract other unwanted compounds in this alga [21]. Hence, 24 seconds was chosen to be the optimal extraction time.

The results of the above study are similar to Abugabr *et al.* when optimizing the protein extraction process from *Eurycoma apiculata* with a microwave power of 270 W to achieve the highest recovery efficiency of 17.15% [22]. Bendin *et al.* also published similar results when collecting protein from rice bran at a microwave time of 40 seconds for a higher recovery efficiency of 22.04% [23].

## 4. CONCLUSION

The research shows the impact of Viscozyme L on the efficiency of lectin extraction, as well as the influence of microwave factors on the extraction process of lectin from *C. aerea* alga. The results show that, for each factor, using microwaves will be more effective in extracting compounds from raw alga than using Viscozyme L. The MAE method resulted in the protein content not being significantly different from the EAE. However, the hemagglutinating activity was 4377.739 ± 68.551 HU/g (1.3 times higher than the specific lectin activity of the EAE method). Thus, the MAE method is considered a more effective and faster method than EAE to extract lectin from *C. aerea* alga.

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## TÓM TẮT

### NGHIÊN CỨU ẢNH HƯỞNG CỦA ENZYME VÀ VI SÓNG ĐẾN TRÍCH LY LECTIN TỪ RONG *Chaetomorpha aerea*

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Lectin thực vật là hợp chất sinh học đầy hứa hẹn và được chú ý khi ứng dụng vào lĩnh vực dược lý, đặc biệt là lectin từ rong tảo. Lectin có nhiều hoạt tính sinh học như kháng oxy hóa, kháng nấm, kháng khuẩn, kháng tế bào ung thư. Rong *Chaetomorpha aerea* chứa nhiều thành phần có hoạt tính sinh học, nổi bật là lectin. Enzyme Viscozyme L và vi sóng được sử dụng để tăng cường hiệu quả chiết xuất lectin từ *C. aerea*. Nghiên cứu này nghiên cứu ảnh hưởng của enzymeViscozyme L và vi sóng đến quá trình chiết xuất lectin từ rong *C. aerea*. Kết quả cho thấy với phương pháp trích ly có sự hỗ trợ của Viscozyme L., hàm lượng protein thu được là 0,098 g/g chất khô và hoạt tính riêng lectin là 3521,075 HU/g. Dưới sự hỗ trợ của vi sóng, hàm lượng protein thu được là 0,101 g/g chất khô và hoạt tính riêng lectin là 4377,739 HU/g.

*Từ khóa: Chaetomorpha aerea*, lectin, trích ly, vi sóng, Viscozyme L.