International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume – 07, Issue – 07, July 2022, PP – 11-15

Study on the extraction and Purification of the red pigment R-phycoerythrin from Gracilariasp

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Abstract: This study reports theextraction and purification of R-Phycoerythrin (R-PE) from red seaweed *Gracilaria sp*. R-PE is a red pigment, a phycobiliprotein of the red algae. It is used for natural colorant in the food and cosmetic products. For freeze-dried algae and soak in phosphate buffer 20mM pH 7.1, presented better results for R-PE extraction after 2 time of extraction. The R-PE yield was 8.2 mg. g^{-1}dw and the Purity Index (PI) of R-PE was 0.19. Then, R-PE purification was performed by the precipitation ammonium sulfate. The concentration of ammoniumsulfate precipitation (90%) and dialyzed 24h has increased the purity index of R-phycoerythrin about 3.8 times than the crude extract (CE).

Keywords: Gracilaria sp., Purification, R-phycoerythrin, crude extract.

I. INTRODUCTION

*Gracilaria sp.*isa genus of the Rhodophyta with agars.Red seaweedis an important role in primary production and are used in food products, cosmeticproducts and pharmacy[1]. The genus Gracilaria were a rich source of agars and they was cultured worldwide today. According the recent researchs, this genus is also a resourcefor extracting and purifying for proteins and R-phycoerythrin. *Gracilaria sp.* was cultivated in China, Taiwan, Korea, Malaysia, Vietnam … [1].

R-phycoerythrin (R-PE) is a phycoerythrins with λmax = 498-565 nm. This pigment is an oligomeric water-soluble chromoprotein which has the subunits including 6α, 6β, 1γ, 1 γ' subunits (about 260kDa) [2]. In the food industry, cosmetics, pharmacy,... R-PE was can be used and applied as natural colorant, fluorescent energy transfer, flow cytometry, fluorescent inmunoassays,… Extraction is an important method for separating different types of values bioactive compounds of seaweeds. However, its efficiency is reduced and depended on presenting of complex cell wall polysaccharides such as cellulose, lignin, agars, alginates, carrageenan... Therefore, different extraction methods have been studied and employed to optimize the extraction efficiency of active compounds (Wijesinghe and Jeon, 2012). Besides, the pretreatment of the material is also important step for extraction these compounds. The classic R-phycoerythrin extraction method is based on maceration in water, distilled water, phosphate buffer, sodium phosphate buffer,.... The pretreated method for the seaweed is freeze-dried, grinding with liquid nitrogen, freezed and defosted, dried algae,.... [3]. The price of this pigment is high (about 380euros/mg) (Sigma Aldrich 2022). R-PE was studied from the seaweed such as: *Gracilaria gracilis, Chondrus crispus, Palmaria palmata, Grateloupia turuturu,...*In this study, we extracted and purified R-PE from *Gracilaria sp*. by soakingin phosphate buffer 20mM pH 7 after pretreated material [4][5] [6].

II. MATERIAL AND METHODS

2.1 Material

Gracilaria sp was collected in the intertidal zone of Thuan An, Vietnam. Epiphytes were removed and washed and cleaned in seawater, tap water and distilled water.

The red algae were pretreated and presevered. We have 5 samples including: wet algae 1(defosted, cut into 1cm), wet algae 2(homogenized in liquid nitrogen), dry algae 1 (solar drying, cut into 2cm), dry algae 2(solar drying, homogenized in liquid nitrogen), the algae powder (freeze-dried and homogenized in liquid nitrogen).

2.2 R-PE extraction and purification

R-phycoerythrin determination

R-PE yield and purity index were determined spectrophotometrically using the method of Beer and Eshel Eq (Beer and Eshel 1985). The absorption spectra of R-PE present three peaks: 498 nm, 545 nm and565 nm. R-PE yield was expressed as mg g^{-1}dw (dry algae)[7].

$$[\text{R-PE}]\ (\text{mg.mL-1}) = [(A_{565}-A_{592}) - (A_{455}-A_{592}) \times 0.20] \times 0.12 \quad \text{(Eq.1)}$$

$$\text{Purity Index or PI} = A_{565}/A_{280} \quad \text{(Eq. 2)}$$

Water-soluble proteins determination

After centrifuged, water-soluble proteins were determined by the method of Bradford (Bradford 1976). Bradford reagent (Sigma) (200 µL) was added to 800 µL of sample solution. The absorbance measurement at 595 nm (read immediately after the reaction) and the use of BSA (Sigma) as a standard (from 0 to 50 mg L^{-1}) enabled the protein content to be determined[8].

Precipitation and dialysis

The supernatant or the crude extract was fractioned with ammonium sulfate from 60% to 100%. The sample and salt allow precipitate to homogenized and stirred for 2 hours at 4°C. Then, recover precipitate by centrifugation, remove supernatant, respin briefly to clear remaining ammonium sulfate. Finally, the precipitate was dissolved in phosphate buffer 20mM pH 7.1 and desalted overnight by dialysis using a Spectra/Por Regenerated Cellulose 3.5 kDa cut-off membrane against phosphate buffer (20 mM, pH 7.1) [5].

III. RESULTS AND DISCUSSION

3.1 Extraction

After pretreating, the samples soaked in phosphate buffer 20 mM, pH 7.1. The results obtained in Fig. 1, Fig. 2. The best results for R-PE yields and proteins contents presented with the powder algae *Gracilaria sp*. (8.21 ± 0.3 mg. g^{-1} dw and 18.89 ± 0.2 mg. g^{-1} dw, respectively).

For the wet algae 1 and wet algae 2, the results were very similar. R-PE yields ranged from 2.1 ± 0.25mg. g^{-1} dw to 2.6. ± 0.20mg. g^{-1} dw. Besides, the protein concentration were from 6.2 ± 0.50mg. g^{-1} dw to 7.1 ± 0.31mg. g^{-1} dw. However, for dry algae 1 (solar drying, cut into 2cm), dry algae 2(solar drying, homogenized in liquid nitrogen) we got samples but they were not pink. After soaking during 20 min, the crude extract had pale yellow. The spectra were not the same as the samples preprocessed by other methods. We can see that the color of seaweed-*Gracilaria sp.* has been lost or denatured after drying with solar energy. In this study, R-PE yields and protein contents were very low. They presented 0.3 ± 0.1mg. g^{-1} dw for R-PE contents and 1.5 ± 0.1mg. g^{-1} dw for protein concentration.

From these results, the algae powder was preferable for R-PE extraction.We could see that the pretreatment of the material is very important. The extracted R-PE content depends on the raw materials and the processing of the raw materials.Then, we performed extractionfive times for the samples of algae powder because we want to enhance the extraction of R-PE yields after soaking by phosphate buffer.

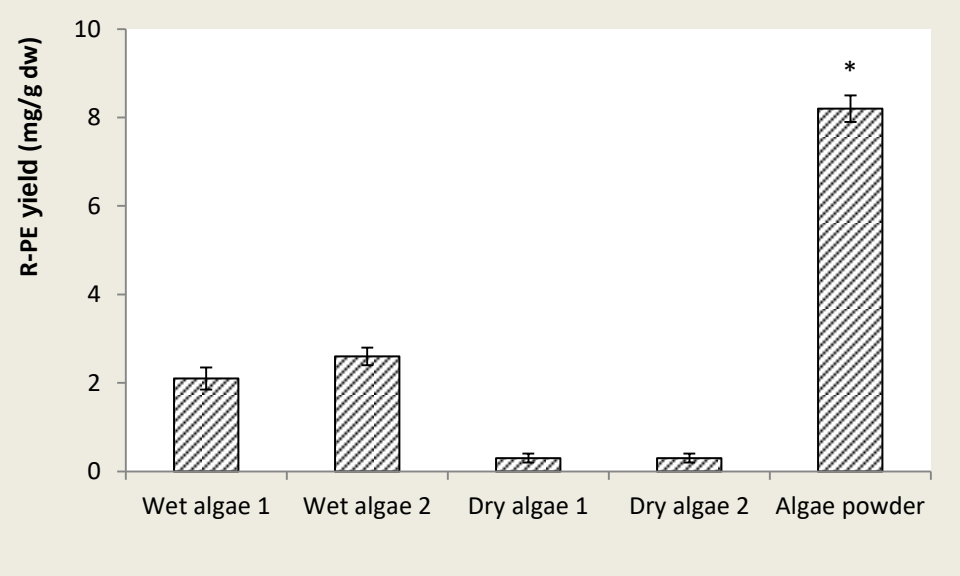

Fig. 1 R-PE yieldsof *Gracilaria sp.* After pretreated the sample and soaking . Values are expressed as the mean ± SD (n=3).

NB: Anova one way significantly different results with p<5% are indicated by *.

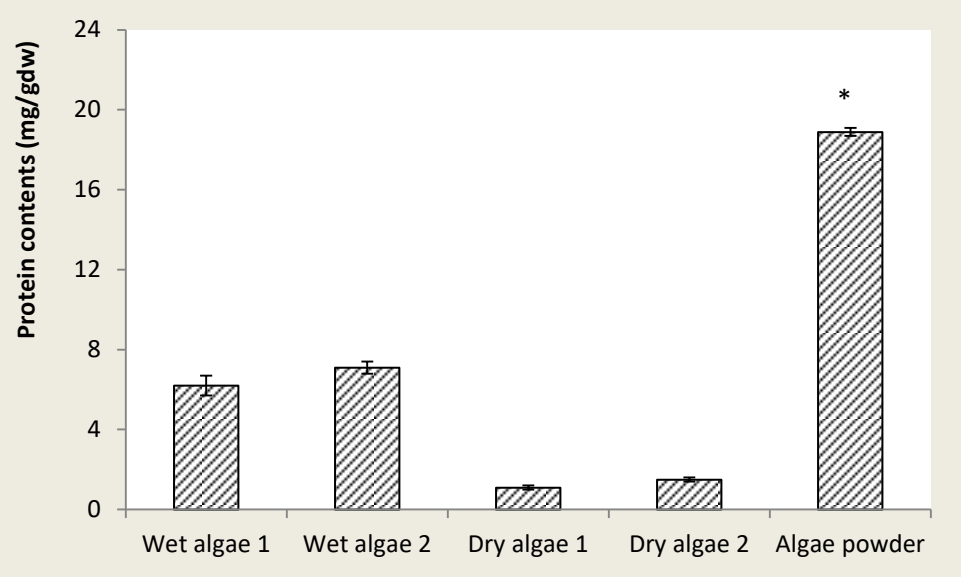

Fig. 2 Protein contents of *Gracilaria sp.* After pretreated the sample and soaking . Values are expressed as the mean ± SD (n=3).

NB: Anova one way significantly different results with p<5% are indicated by *.

From the algae powder, after centrifuged and obtained the crude extract, the residue will be extracted repeatedly. Then , after five times soaking andextraction, the yield of R-phycoerythrin of CE were presented (Fig.3).The R-Phycoerythrin yield after first soaking and extracting (during 20min) obtained the highest value, 8,2 ± 0.3 mg. g^{-1} dw. In addition, the R-phycoerythrin yields (Fig.3) show that there is a significant difference between the yields of the first step (EX1), and the yield of steps 2,3, 4, 5 (EX2, EX3, EX4, EX5) in all five extraction ($p<0.05$). The extraction yields of the R-PE after the threesoakingare low. For the pigment, during 20 min of the first extraction, it was observed that the supernatants of the extractions EX1 and EX2 have a pink. However, the samples EX4 and EX5 have non-pink color, displaying a denaturation of this pigment, as revealed by the data illustrated in Fig.3.

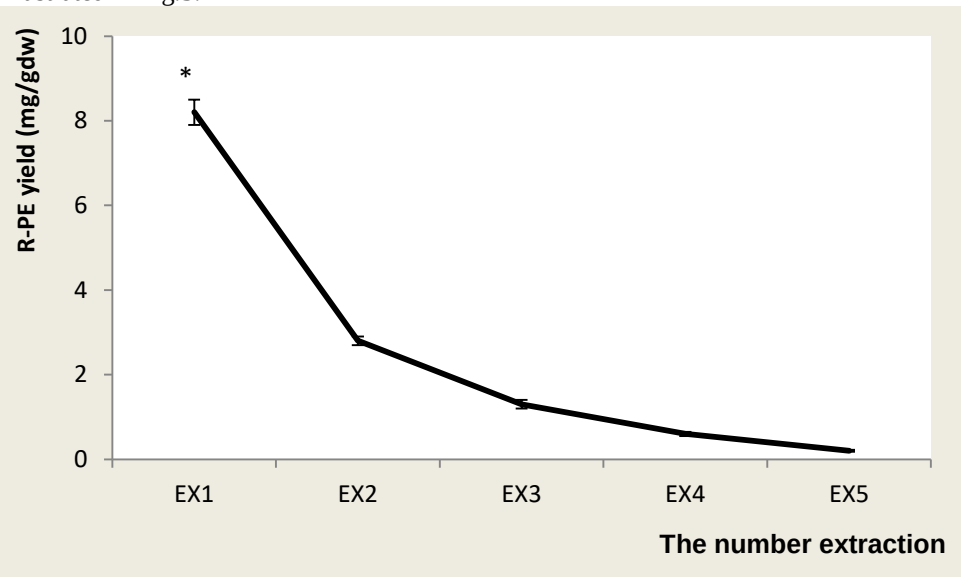

Fig. 3. The numbers of extraction (EX): 5 times (phosphate buffer 20 mM, pH 7.1) and R-PE yield

NB: Anova one way significantly different results with p<5% are indicated by *.

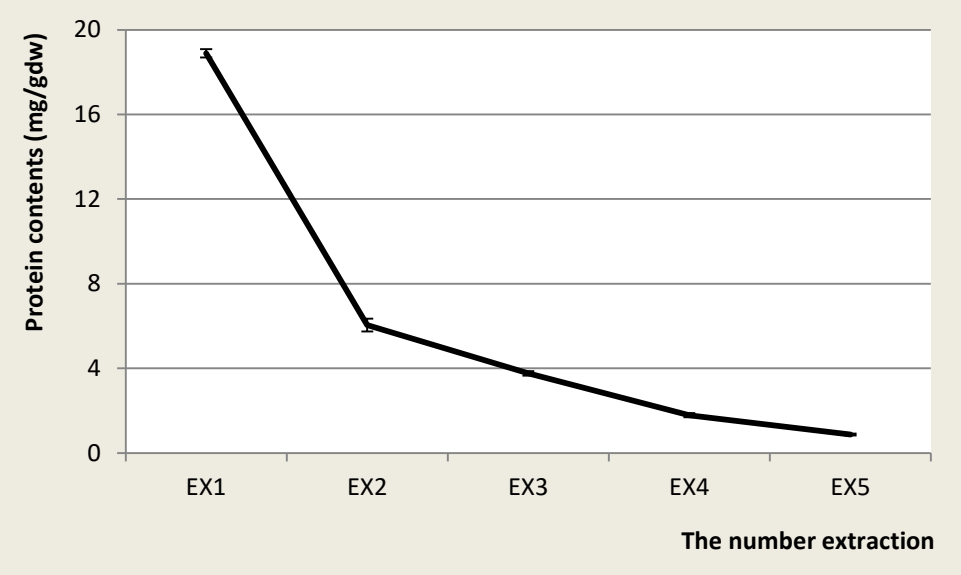

Fig. 4.The numbers of extraction (EX): 5 times (phosphate buffer 20 mM, pH 7.1) and proteint contents

NB: Anova one way significantly different results with p<5% are indicated by *.

For enhancing the extraction R-phycoerythrin from the seaweed, the benefits for pretreatment of the materials were demonstrated by several researchs [3].They determined that pretreatment materials could improve and enhance for extraction process, especially freeze-dried methode. Freeze –dried seaweed and grinding samples in liquid nitrogen tend to easier to extract R-PE and proteins. However, solar drying of algae has discolored and denatured the protein as well as the R-PE. This can be explained under the effect of sunlight, the prolonged drying time has lost the natural color of the algae.

Precipitation ammonium sulfate and Dialysis

In this study, the crude extract of R-PE after soaking in phosphat buffer for powder algae, and then centrifuged. The precipitation ammonium sulfate and dialysis were used to purify the R-phycoerythrin of *Gracilariasp.* The concentration of ammonium sulfate precipitation performed from 60-100% of saturation) [5]. After centrifuged and dialysis, we determined the purity of the R-PE (Fig 5).

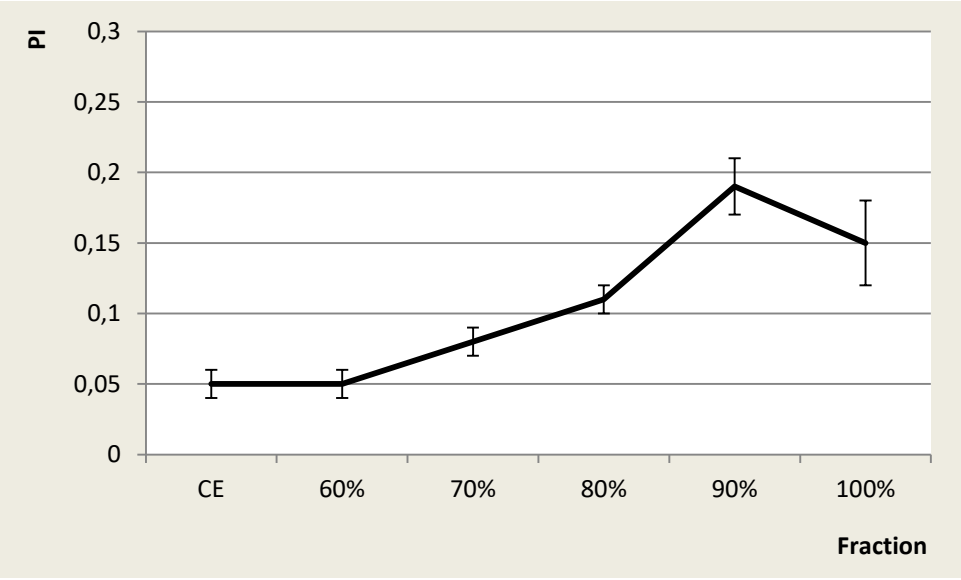

Fig 5.R-phycoerythrin purity of the different fractions from *Gracilaria sp.* with the ammonium sulfate 60-100% saturation. Values are expressed as the mean ± SD (n=3)

From the Fig 4, the results presented that the purity index of the pigment R-phycoerythrin for the samples after precipitated by ammonium sulfate and dialysized 24h were higher than the crude extract (CE). The concentration of ammonium sulfate precipitation (90%) has increased the purity index of R-PE about 3.8 times than the crude extract.

However, the ratioA_{565}/A_{280} of the pigment R-PE after precipitated by ammonium sulfate precipitation and dialyzed was still low. These results are completely consistent with previous studies for this method and *Gracilaria verrucosa* [9]. Consequently, the futher research can continue others methods such as the chromatography, ultra filtre to purify for this pigment R-phycoerythrin and water solubles proteins.

IV. CONCLUSION

In this study, we can confirm that pretreatment of the material couldimprove and enhance R-PE yield from *Gracilaria sp*. Freezed-dried is one of methods which offers high R-PE extraction efficiency compared to other methods. Especially freeze-dried algae and grinding with liquid nitrogen could be increased and improved Rphycoerythrin concentration. In this future work, we can be present about the structure of R-phycoerythrin from *Gracilaria sp* by SDS-PAGES. Besides, we will also research to be stable the natural pigment R-phycoerythrin for applications form the seaweed *Gracilaria sp*.

ACKNOWLEDGEMENTS

This research was supported from the University of Technology and Education, University of Danang, Vietnam.

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