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RESEARCH ON USING ADVANCED TECHNIQUES AND INTEGRATED TECHNOLOGY FOR COMPREHENSIVE PROCESSING BROWN SEAWEED INTO VALUABLE PRODUCTS

SUMMARY OF DISSERTATION ON CHEMICAL ENGINEERING

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Tran Quoc Toan, **Tran Duy Phong**, Dam Duc Tien, Nguyen Manh Linh, Nguyen Thi Mai Anh, Pham Thi Hong Minh, Le Xuan Duy, Do Huu Nghi, Hai Ha Pham Thi, Pham Tri Nhut, Ho Sy Tung, and Nguyen Quang Tung, 2021, Optimization of Microwave-Assisted Extraction of Phlorotannin From *Sargassum swartzii* (Turn.) C. Ag. With Etanol/Water, *Natural Product Communications*, 16 (2), pp. 1-11.
2. Trần Quốc Toàn, Phạm Quốc Long, Hoàng Thị Bích, **Trần Duy Phong**, Phạm Minh Quân, GPHI thuộc Bằng độc quyền Giải pháp hữu ích số 2606 *"Quy trình chế biến rong Mơ (Sargassum.SP) để thu các sản phẩm Fucoxanthin, Phlorotanin, Fucoidan và Alginate theo phương pháp sử dụng sóng siêu âm cường độ cao kết hợp enzym"*, cấp theo quyết định số 3750w/QĐ-SHTT, 08/3/2021, Cục sở hữu trí tuệ Việt Nam.
3. Đặng Thị Phương Ly, **Trần Duy Phong**, Trần Quốc Toàn, Đoàn Lan Phương, Trịnh Thu Hương, Đặng Thị Minh Tuyết, Đào Thị Kim Dung, Lại Phương Phương Thảo, Hoàng Thị Bích, Phạm Minh Quân, Đàm Đức Tiến, Lưu Văn Huyền, Phạm Quốc Long, 2019, Bước đầu đánh giá hàm lượng Lipid và thành phần Axit béo của một số loài rong Nâu khu vực biển Bắc và trung Trung Bộ - Việt Nam, *Tuyển tập hội nghị khoa học biển,* tr. 579-585.
4. Trịnh Thị Thu Hương, Đào Thị Kim Dung, Phạm Thu Huế, Lê Tất Thành, Đỗ Thị Thảo, Nguyễn Thị Cúc, Phạm Minh Quân, Trần Quốc Toàn, **Trần Duy Phong,** Phạm Quốc Long, 2019, Đánh giá chỉ tiêu sinh hóa và hoạt tính chống loãng xương của hoạt chất canxi alginate từ nguồn rong biển Việt Nam, *Tuyển tập hội nghị khoa học biển*, tr. 572-578.

PREAMBLE

1. The urgency of the thesis

Brown seaweed is determined to have many valuable ingredients such as alginic acid, fucoidan, fucoxanthin and phlorotanin... However, the exploitation and processing of Brown seaweed in Vietnam is currently limited, Brown seaweed is mainly processed into food at the household scale. The extraction of valuable products from Brown seaweed has only stopped at a laboratory or pilot scale. Some studies have applied advanced techniques (ultrasonic extraction, enzyme-microbiological extraction, extraction using microwave...) but only stopped at extracting specific ingredients, not yet providing a comprehensive processing process for Brown seaweed.

2. Research objectives of the thesis

Research and integrated use of advanced techniques such as ultrasound, microwaves, enzymes, high-speed centrifugation... to build a comprehensive technological process for processing Brown seaweed into high-value products (phenolic, fucoidan, alginate...). By-products of the processing process will be produced into microbial fertilizer. Then we will evaluate the quality and some biological activities of the Calcium alginate powder preparation.

3. Main research contents of the thesis

- This study will investigate the alginate content, fatty acid content and lipid class, and predict the ability to inhibit the Tyrosinade enzyme of some compounds extracted from Brown seaweed using the virtual screening method *(in silico docking)*.

- The thesis will build a comprehensive technological process for processing Brown seaweed into high-value products such as phenolic, fucoidan and alginate using advanced techniques (microwaves, enzymes, ultrasound, high-speed centrifugation…). Waste from the processing process will be researched as fertilizer. We will evaluate the quality and biological activity of the Calcium alginate product.

- We will use response surface method to optimize phenolic extraction technology from Brown seaweed using microwave assisted method.

CHAPTER 1. OVERVIEW

1.1. Introducing Brown seaweed

1.1.1. General introduction

The phylum Brown algae (Phaeophyta or Ochrophyta), includes the class Phaeophyceae, including multicellular brown algae with many different morphologies and sizes, including 16 orders with about 285 genera and about 2040 species, of which about 1500 species have been discovered. identified worldwide. Except for a few freshwater genera (less than 1%), most brown seaweeds are marine and largely grow in subtidal areas. Of these, 95% of Brown seaweed species are widely distributed in cold to temperate waters.

As of 2013, 827 species have been recorded in Vietnam, belonging to 4 phyla: Blue seaweed (Cyanophyta), Red seaweed (Rhodophyta), Brown seaweed (Ochrophyta) and Green seaweed (Chlorophyta). Up to now, there has been no work to assess the total seaweed reserves in the entire waters of Vietnam because studies are usually only conducted in each separate area and only present the reserves of a few seaweed species economy at the time of the study. Some programs to investigate and evaluate seaweed resources put the reserves of *Sargassum*, a member of the Brown seaweed family, at about 35,000 - 75,000 tons.

1.1.2. Chemical composition, activity and applications

Like other seaweeds, Brown seaweed contains basic substances such as carbohydrates (4-70% dry matter), proteins (3-24% dry matter), lipids (0.3-4.8% dry matter), ash (14-45% dry matter). Seaweed in general and Brown seaweed in particular contain highly biologically active compounds such as pigments (carotenoids), polysaccharides (alginate, fucoidan), storage lipids, vitamins,... Therefore, they has potential applications in the fields of medicine, food, industry...

1.2. Brown seaweed processing technologies

1.2.1. Traditional technology

1.2.2. Modern technology

- Ultrasound assisted extraction (UAE)
- Enzyme assisted extraction (EAE)
- Microwave assisted extraction (MAE)
- Some other modern methods:

+ Pressurized liquid extraction (PLE)
+ Extrusion assisted extraction (ExEA)
+ Supercritical fluid extraction (SFE)

1.3. Research orientation

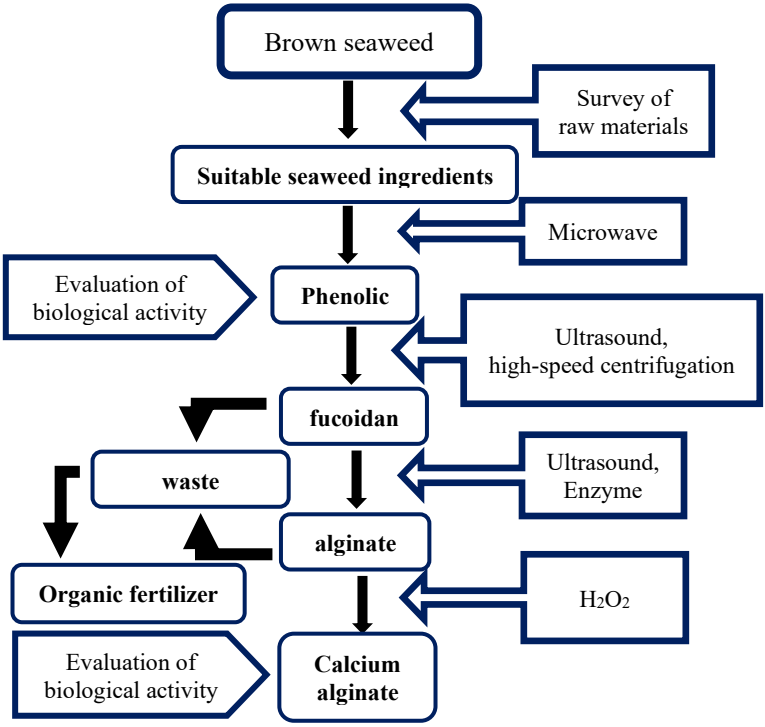

Figure 1.1. Research orientation diagram

CHAPTER 2. RESEARCH SUBJECTS AND METHODS

2.1. Materials

Brown seaweed samples were collected in some Vietnamese waters using direct sampling and deep diving methods. This work must be carried out by diving scientists with appropriate qualifications. After collection, the seaweed samples were removed and washed with sea water, then dried to a humidity below 35% and stored at the Institute of Natural Products Chemistry before processing. Samples were identified by Associate Professor. Dr. Dam Duc Tien, Institute of Marine Resources and Environment.

We use chemicals and research equipment from the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

2.2. Research Methods

2.2.1. Method for determining total phenolic content

2.2.2. Method for determining alginate

2.2.3. Virtual screening method (in silico docking)

2.2.4. Method for determining lipid content and composition

2.2.5. Method for determining the composition and content of fatty acids

2.2.6. Determination of Fucoidan content by colorimetric method

2.2.7. Determination of fucoxanthin content

2.3. Methods to evaluate biological effects

2.3.1. Method for evaluating safety indicators

2.3.2. Method for determining acute toxicity

2.3.3. Study on semi-chronic toxicity

2.3.4. Method to evaluate the effect of heavy metal elimination

2.3.5. Method to evaluate the ability to prevent osteoporosis

2.3.6. Test method for antioxidant activity

2.3.7. Test method for cytotoxic activity

CHAPTER 3. RESULTS AND DISCUSSION

3. 1. Results of research and survey of some raw Brown seaweed samples

3.1.1. Research and survey of alginate content of some types of Brown seaweed harvested in Vietnam's sea

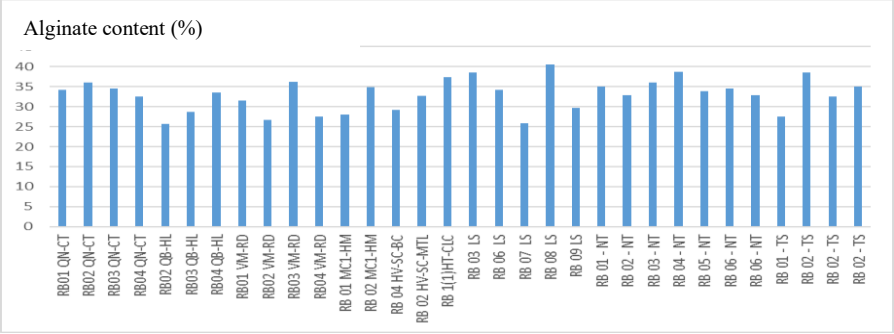

Figure 3.1. Alginate content in some Brown seaweed samples

Through research about alginate content and quantity of Brown seaweeds, we can choose *S. Mcclurei, S. swartzii* and *S. polycystum* (*Sargassum* genus) to conduct alginate extraction studies.

Table 3.1. Main chemical components of some types of seaweed

Num	Name	Main chemical composition (%)						
		Protein	Lipit	Tro	Sulfat	Alginate	Laminaran	Fucoidan
1	*S. mcclurei*	5.5	1.9	37.8	4.9	32.1	0.08	2.4
2	*S. swartzii*	7.0	0.5	30.2	5.8	29.5	0.29	0.82
3	*S. polycystum*	3.1	0.6	46.3	3.9	29.7	0	2.7

3.1.2. Research to investigate the content of fatty acids and lipid classes

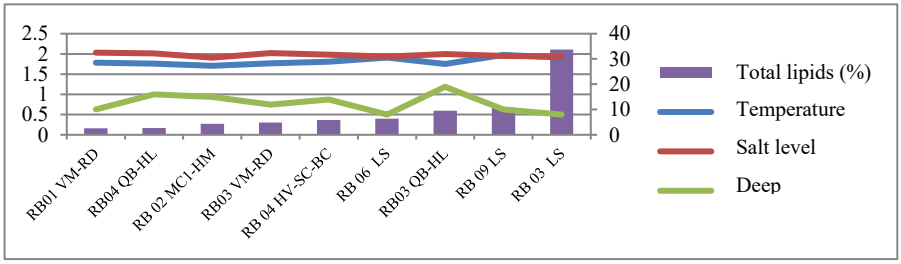

Figure 3.2. Results on total lipid content of Brown seaweed samples

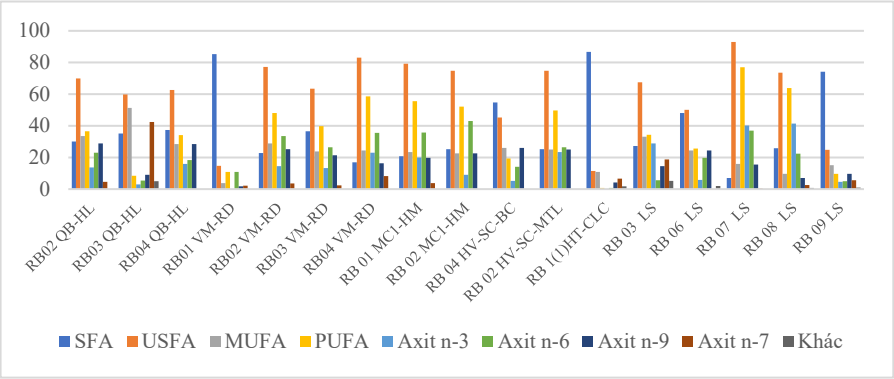

Figure 3.3. Fatty acid content in Brown seaweed samples was surveyed

3.1.3. Research predicts the skin whitening ability of some compounds isolated from Sargassum seaweed

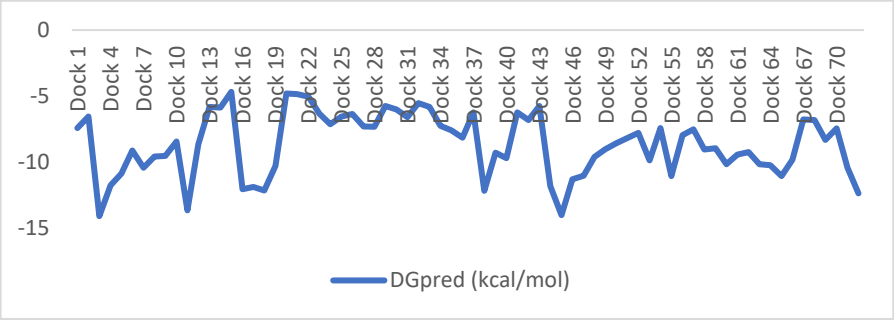

Figure 3.4. Results predict the binding energy of compounds with tyrosinase enzyme

The results of data synthesis show that compounds 3, 11, 16, 18, 38 and 45 were determined to have the potential to create strong binding affinity with the enzyme tyrosinase. In particular, the binding free energy of three substances 3, 11 and 45 exceeds the value obtained with the standard ligand tropolone.

Two-dimensional and three-dimensional interaction imaging results of compounds 3, 11 and 45 show that, among the three compounds showing strong binding affinity to sEH, only compounds 11 and 45 have potential Inhibits the activity of this enzyme through direct interactions with important

amino acids. Compound 3 was observed not to form hydrogen bonds with the enzyme tyrosinase. Therefore, it can be concluded that compound 3 is not a potential inhibitor of the studied receptor target.

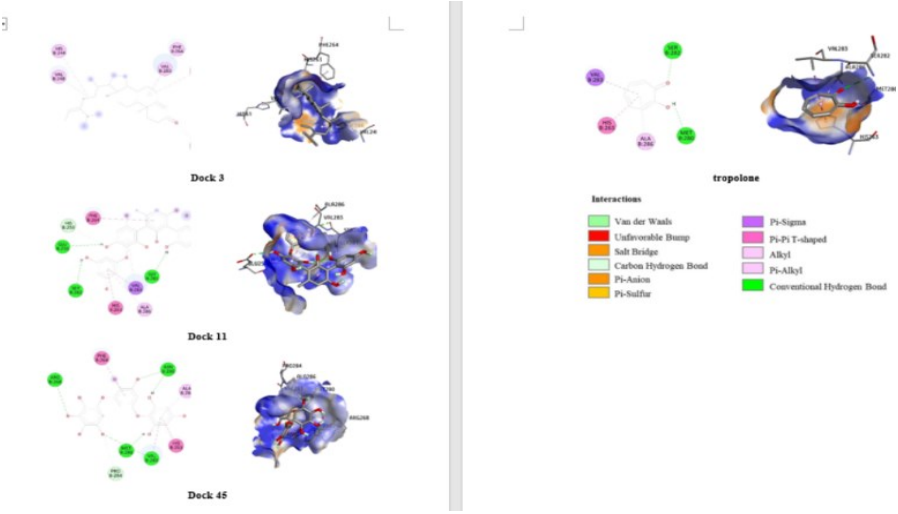

Figure 3.5. Binding configurations in two-dimensional and three-dimensional space were predicted by AutoDock4.2.6 software of the compounds

Compounds 11 and 45 have HIA values of 0.9897 and 0.8692, respectively. This shows high absorption in the intestinal tract. It can be said that compound 11 has higher potential in drug development than compound 45 thanks to its superiority in ADMET indexes.

Table 3.2. Predicting the toxicity of potential inhibitors

Com-pounds	MW	HBD	HBA	LogP	MR (cm^3/mol)	LD_{50} (mg/kg)	Toxicity prediction[a]	HIA
Dock 11	496	10	11	4.17	125.21	600	4	0.9897
Dock 45	406	9	11	2.19	94.24	550	4	0.8692
tropolone	122	1	2	1.12	33.99	385	4	0.9923

3.1.4. Selecting seaweed raw materials for technological processes

Through research results and surveys of some samples of Brown seaweed materials, it can be seen: (1) The Sargassum genus of the Brown

seaweed industry appears mostly in Vietnamese waters, with average alginate content. average up to over 30%, (2) The Sargassum genus have total lipid content from 0.07 to 2.11%; all contain saturated fatty acids, monounsaturated fatty acids and especially polyunsaturated fatty acids (PUFA); (3) Compound No. 11 (difucodiphlorethol A) isolated from the Sargassum genus has potential in developing tyrosinase enzyme inhibitors.

The research orientation focuses on extracting alginate to create Calcium alginate product, and at the same time aims to apply the process to production on an industrial scale (requiring large raw material reserves), so the seaweed sample *S .swartzii* collected in Co To, Quang Ninh (2019) was selected for further research.

3.2. Technique for extracting phenolics

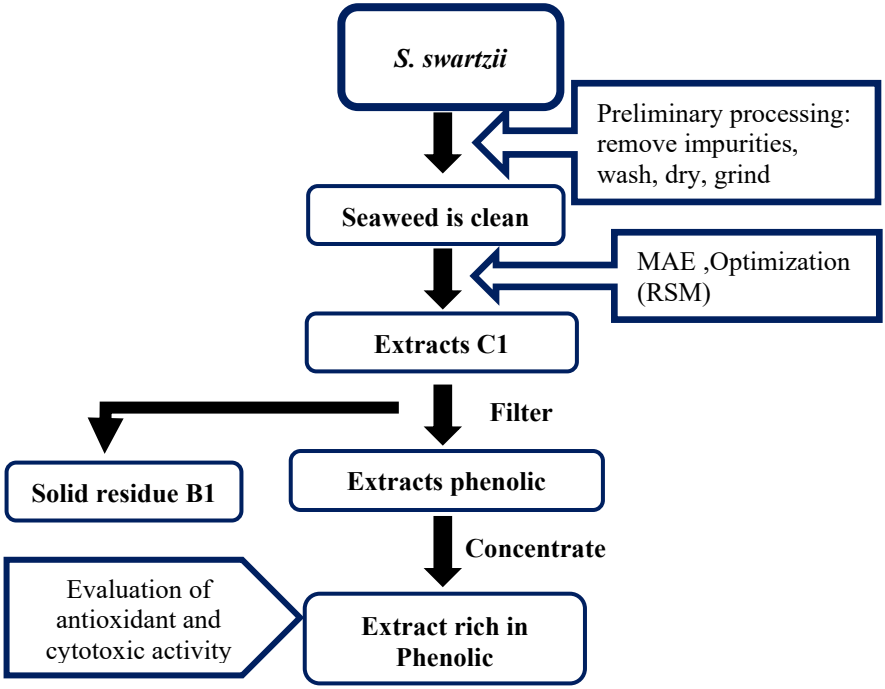

Figure 3.6. Phenolic extraction diagram

3.2.1. Results of research on factors affecting the extraction of phenolics from Brown seaweed using the microwave extraction method

Conducting experiments to investigate factors affecting the extraction of phenolics from *S .swartzii* are: solvent concentration (ethanol 0%, 30%, 50%, 75%, 96%); solvent/material ratio (15/1, 20/1, 25/1, 30/1, 35/1, 40/1); extraction time (15, 30, 45,60, 75, 90 minutes); microwave power (80, 240, 400, 560, 800 W).

Selection results: Ethanol concentration 60%, solvent/material ratio 35/1, extraction time 60 minutes, extraction power 400W.

3.2.2. Results of optimizing phenolic extraction conditions using response surface method (RSM)

The phenolic content of the extract is predicted to be 1.52 mgGAE/g, the optimal extract mass is predicted to be 8.6 mg. The optimal parameters of the extraction process are predicted to be: ethanol concentration 54.5%; Solvent/ material ratio is 34.6/1; Extraction time is 64.28 minutes and microwave power is 473.6W. Conduct extraction experiments at 55% ethanol concentration conditions; ratio of solvent/material = 35/1; Extraction time is 65 minutes and microwave power is 475W and evaluation of phenolic content and extracted extract mass shows: Experimentally obtained phenolic content is 1.50 ± 0.12 mgGAE/g; The extracted mass was 8.52 ± 0.11 mg, which was not significantly different from the prediction.

3.2.3 Evaluation of the activity of phenolic extracts

Table 3.3. Results of antioxidant activity assessment

Num	Sample for experiment	SC_{50} (µg/mL)
1	Control (+) [ascorbic acid]	12.6
2	Control (-) [DPPH/EtOH+ DMSO]	-
3	Samples processed by MAE	683
4	Samples processed by UAE	590

Table 3.4. Results of evaluating the cytotoxic activity of phenolic extracts

Num	Sample for experiment	IC_{50}, µg/mL		
		MCF-7	HeLa	PC3
1	Paclitaxel	0.38	0.55	0.34
2	Samples processed by UAE	12.80	27.83	57.40
3	Samples processed by MAE	12.78	42.68	67.25

The results showed that the phenolic extract sample had moderate antioxidant and cytotoxic activity, and the phenolic extract sample using the ultrasound method had better activity than the microwave sample.

3.3. Research on fucoidan extraction process

3.3.1. Technological process for extracting fucoidan from seaweed residue

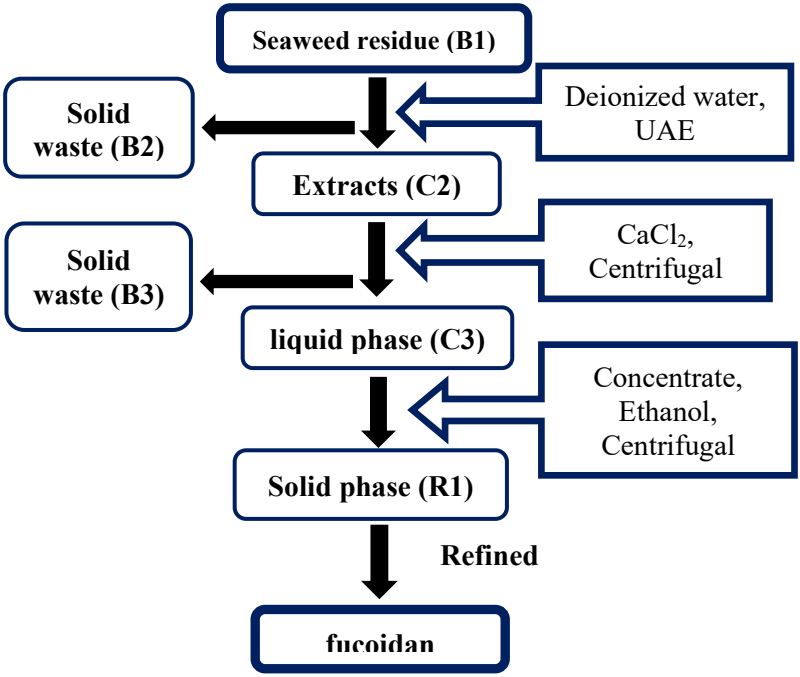

Figure 3.7. Diagram of fucoidan extraction from seaweed residue

After phenolic extraction, the remaining seaweed residue has a negligible decrease in alginate, protein and ash content because these substances are insoluble in ethanol, so this seaweed residue can be used to extract fucoidan and alginate.

Process explanation

The B1 solid residue (obtained after phenolic extraction) will be processed to extract fucoidan.

Step 1: B1 solid mixture is added with deionized water in a specified ratio, stirred well, then sonicated at different power, time and temperature values, filtered and obtained the filtrate C2, solid fraction B2.

Step 2: The filtrate C2 is added with $CaCl_2$, centrifuging and take the solid fraction B3 and the liquid phase C3.

Step 3: The liquid phase C3 is evaporated under reduced pressure. Add ethanol 96^o at a specified ratio, centrifuge at high speed to obtain solid phase R1. This solid fraction is purified to obtain fucoidan.

3.3.2. Research results on fucoidan absorption process

Conducting a survey of factors affecting the fucoidan extraction process by ultrasonic method, including: **(i)** Determining the solvent for fucoidan extraction (HCl, deionized water, CaCl2); **(ii)** Effect of solvent: raw material ratio (6:1, 7:1, 8:1, 9:1 ml/g); (iii) Effects of temperature (25, 30, 40, 50, 60, 70^{o}C) time (0, 1, 2, 3, 4, 5, 6 minutes) and ultrasound intensity (40, 50, 58W/cm^2); **(iv)** Effect of $CaCl_2$ content on fucoidan purity (0.25; 0.50; 0.75, 1.00, 1.25 g $CaCl_2$/liter of extract); **(v)** Effect of ethanol concentration on the ability to precipitate fucoidan from the filtrate (50, 55, 60, 65, 70%).

Research results show that: (i) Reduced water was chosen as the solvent to extract fucoidan from seaweed using ultrasound because it is easy to produce, non-toxic, low cost and no difference in performance compared to HCl; **(ii)** The solvent/ material ratio is 8/1 (ml/g) suitable for extracting fucoidan from seaweed residue using ultrasound waves, helping to ensure process efficiency as well as optimize economic efficiency (reduce costs of

raw materials, energy...); **(iii)** The parameters of the ultrasound process to extract fucoidan from seaweed are: 40°C, ultrasound intensity 58W/cm^2, 3 minutes; **(iv)** The $CaCl_2$ content to precipitate alginic acid in the extract is 0.75g $CaCl_2$/liter of extract; **(v)** Ethanol concentration of 60% is suitable to precipitate and recover the active ingredient fucoidan from the filtrate.

3.4. Research on alginate extraction techniques

3.4.1. Technological process of alginate extraction

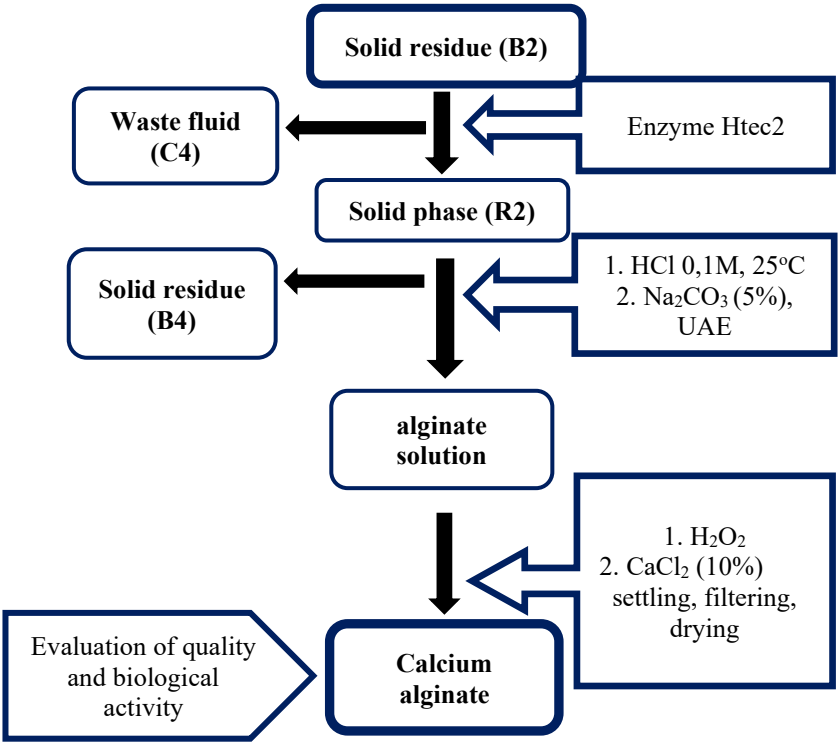

Figure 3.8. Diagram of alginate extraction

After extracting fucoidan, the alginate content in seaweed residue does not decrease significantly, so this seaweed residue can be used to extract alginate.

Process explanation

Step 1: The solid residue B2 is supplemented with Htec2 enzyme in a specified ratio (enzyme/substrate) at pH=5, carried out for a specified time

and temperature, then cool the mixture to room temperature. After enzymatic treatment, we obtain solid phase R2 and waste fluid (C4).

Step 2: Solid residuephase R2 is soaked in 0.1M HCl solution at 25°C, filter the mixture to collect the solid part, wash with clean water, then add NaCl 5% and ultrasound. The resulting mixture was cooled to room temperature, then filtered to collect the crude algenate solution and B4 solid residue.

Step 3: The crude algenate solution is added slowly H2O2 5% solution, stirred for 15 minutes; affter that, added slowly CaCl2 10% solution, allowed to settle for 2 hours, then filtered and dried to obtain the calcium alginate product.

3.4.2. Research results on the hydrolysis of materials by enzymes

Table 3.5. Investigate the effects of treating raw materials with enzymes

Enzyme	Reducing sugar (µg/ml)	Alginate (%)	Viscosity (1%) (*mPa.S*)
Control sample	0	51.43 ± 0.24	235
Raw seaweed + enzyme	27.13 ± 0,32	59.21 ± 0.21	230
Seaweed extract	0	63.32 ± 0.15	238
Seaweed extract + enzyme	**55.25** ± 0,24	**75.01 ± 0.24**	**233**

Enzymatic pretreatment of raw materials significantly increased alginate recovery efficiency. The following conditions are best for cell wall cellulose degradation using Htec2 enzyme: pH is 4.5-5.0, temperature is 45°C, Htec2/substrate concentration was 1.5%, time is 6-8 hours.

3.4.3. Survey of factors affecting the alginate extraction process

Research on factors affecting the alginate extraction process (performance and viscosity) after enzyme treatment, including: **(i)** Effect of HCl concentration (0.0; ; 0.05; 0.1 ; 0.15; 0.2M) and Na_2CO_3 5% additional ratio compared to raw materials (1; 1.5; 2; 2.5; 3%) to treat seaweed residue; **(ii)** Effects of temperature (30, 40, 50, 60, 70, 80, 90, 100°C), time (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 minutes) and ultrasound intensity (0, 40, 50, 58W/cm^2).

Research results show that the appropriate parameters for the process of extracting alginate from seaweed residue using the ultrasonic method are: **(i)** The material is treated with HCl at a concentration of 0.1M at 25°C; affter that, add a 2% amount of 5% Na_2CO_3 solution; **(ii)** Extract alginate by ultrasound at temperature 60°C, ultrasound intensity 58 W/cm^2 for 6 minutes.

3.4.4 Research the process of obtaining Calcium alginate produc

Investigate factors affecting the process of obtaining Calcium alginate products, including: **(i)** Effect of 5% H_2O_2 solution ratio on alginate bleaching process (addition ratio compared to raw material is 0.5, 0.75, 1.00, 1.25, 2.00, 2.25, 2.50, 2.75%); **(ii)** Effect of 10% $CaCl_2$ ratio on calcium alginate powder recovery efficiency ($CaCl_2$/material: 1/1, 1.25/1, 1.5/1, 1.75/1, 2/1 , 2.25/1, 2.5/1 ml/ml).

Research results show that: (i) Bleaching with 5% H_2O_2 solution with an equivalent amount of 2% H_2O_2 compared to NaAlg is appropriate; **(ii)** Add 10% $CaCl_2$ concentration with the corresponding ratio of $CaCl_2$/ bleached alginate solution = 2/1 (ml/ml) to precipitate and recover $Ca(Alg)_2$ from NaAlg.

3.5. Evaluation of the quality and some activities of Calcium alginate

3.5.1. Evaluation of the quality of calcium alginate

Table 3.6. Identify key quality indicators

Num	Target name	Unit	Quality level
1	Humidity	%	6.82
2	Total ash content	%	31.08
3	Calcium content	%	7-10
4	Alginic content	%	20.67
5	pH		6,0.7,5
6	Viscosity (1%)	(*mPa.S*)	235

The research sample has a moisture value of 6.82%; Ash content reaches 31.08% and Alginic content reaches 20.67%. Besides, the calcium content in alginate powder is very high from 7-10%, showing that alginate powder is very good for bones and joints.

Table 3.7. Results of analysis of microbial indicators

Num	Target name	Unit	Quality level	Quality level (QCVN*)
1	Total number of aerobic microorganisms	CFU/g	≤ 1000	≤ 5000
2	Total number of mold and yeast spores	CFU/g	≤100	≤ 500
3	*Coliform*	CFU/g	≤10	
4	*E. coli*	CFU/g	Negative	Negative
5	*S. aureus*	CFU/g	≤ 3	
6	*C. perfringens*	CFU/g	≤ 10	
7	*B. Cereus*	CFU/g	≤ 10	
8	*Salmonella*	CFU/25g	Negative	Negative

* Quality level according to Vietnamese standards

The results of the analysis of microbial criteria showed that in the research sample, *Coliform bacteria, S. Aureus, C. Perfringens, B. Cereus* were present with a content of ≤10CFU/g and two types of bacteria were absent. diseases are *E. Coli* and *Salmonella*. This result meets food safety standards issued by the Vietnam Ministry of Health.

Table 3.8. Heavy metal content criteria

Num	Target name	Unit	Quality level	Quality level (QCVN*)
1	Pb content	ppm	≤3,0	≤5,0
2	Cd content	ppm	≤0,1	≤3,0
3	Hg content	ppm	≤1,0	≤1,0
4	Copper content	ppm	≤10	
5	Zinc content	ppm	≤10	

* Quality level according to Vietnamese standards

Cu and Zn content have values ≤10 ppm; The content of heavy metals Pb, Cd, and Hg is much lower. The values of heavy metal content are within the allowable threshold according to food safety regulations of the Vietnam Ministry of Health.

3.5.2. Evaluation of anti-osteoporosis activity

Calcium alginate at a concentration of 20 µg/ml showed the ability to enhance collagen synthesis compared to the negative control, the percentage of collagen synthesized was 111.10%. At a concentration of 4 µg/ml, the test sample had the ability to stimulate mineral formation slightly compared to the negative control, the percentage of minerals synthesized was 115.42% (P<0.05).

3.5.3. Results of safety research

- Gelalginate preparation does not cause acute toxicity to mice orally with a maximum tested dose of 2.5 g/kg body weight.

Figure 3.9. Photo of mice after acute toxicity experiment

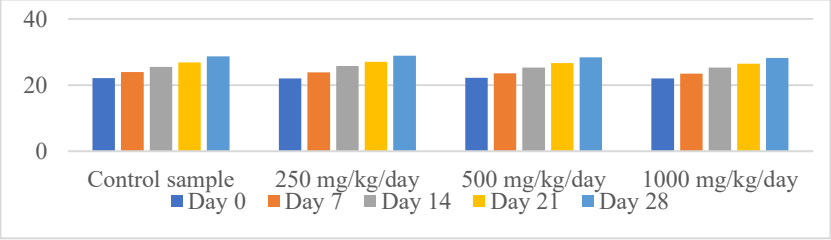

Figure 3.10. Change in mouse weight after giving calcium alginate

- Gelalginate preparation at a dose of 500 mg/kg/day, given semi-chronically for 28 days: The product did not affect the weight gain of experimental mice compared to the control (p>0.05); did not affect hematological parameters compared to the control group (p>0.05); No effect on liver, kidney, spleen weight compared to control (p>0.05); did not

increase liver AST and ALT index compared to control; did not affect basic kidney enzyme index compared to the control group (p>0.05).

- Gelalginate product at dose of 1000 mg/kg/day, given semi-chronically for 28 days: The product did not affect the weight gain of experimental mice compared to the control (p>0.05); did not affect other hematological parameters compared to the control group (p>0.05); No effect on liver, kidney, spleen weight compared to control (p>0.05); did not increase liver AST and ALT index compared to control.

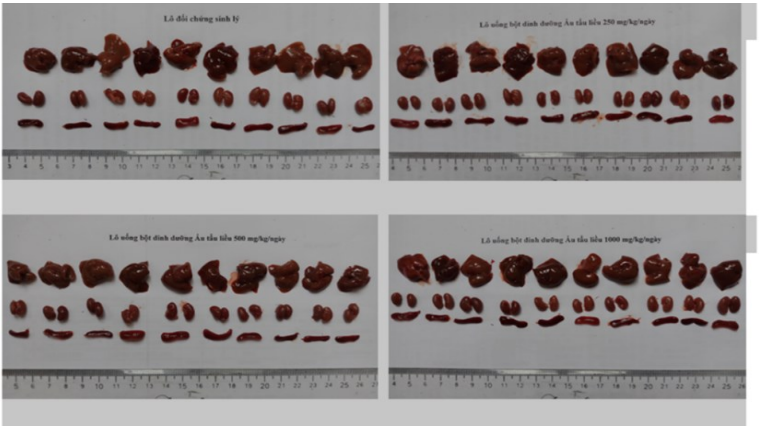

Figure 3.11. Images of liver, kidney, and spleen of mice after semi-chronic toxicity experiment

3.5.4. Evaluation of the heavy metal removal effect of alginate preparations

Calcium algiante is effective in supporting heavy metal excretion in animals with heavy metal poisoning. Using calcium alginate continuously for 9 weeks at a dose of 0.1g/kg body weight/day helps eliminate heavy metal content from the animal's body by 60 - 70% depending on the specific part. Timely use of calcium alginate salt immediately after heavy metal poisoning will result in more effective detoxification.

3.6. Research on treatment of by-products of the processing process

By-products of seaweed processing after alginate extraction are mainly seaweed residue and some waste. These ingredients have a quite high

pH (~ 10), so we process them using microbial products to ferment to create organic fertilizer.

Table 3.9. Effect of inoculant type on C/N ratio of compost

TT	Name	C_{TS}	N_{TS}	C/N
1	No added products	67.23	1.34	50.17
2	EMZEO	39.54	2.56	15.45
3	EM-fert 1	39.77	2.67	14.90
4	S.EM	37.12	3.01	12.33

The S.EM product has the best C/N ratio of 12.33 and was selected for further research.

Results of monitoring the process of composting seaweed residue with S.EM product show that: After 6 weeks of composting, the seaweed residue after extracting alginate and fuicoidan has a brown, slightly yellow color and turns into black, loose organic fertilizer. spongy, soft to the touch, no unpleasant odor.

(a) **(b)**

Figure 3.12. Seaweed residue before composting (a) and after composting (b)

Under the influence of the microflora in S.EM preparation, the physicochemical properties of seaweed residue change. OC content decreased, N_{TS}, P_2O_5 and K_2O increase. Thus, the addition of useful microorganisms is essential for the composting process, increasing the ability to decompose organic compounds in seaweed residue into humus.

3.7. 3.7. Building an integrated technological process for processing products from Sargassum seaweed

3.7.1. Process diagram and explanation

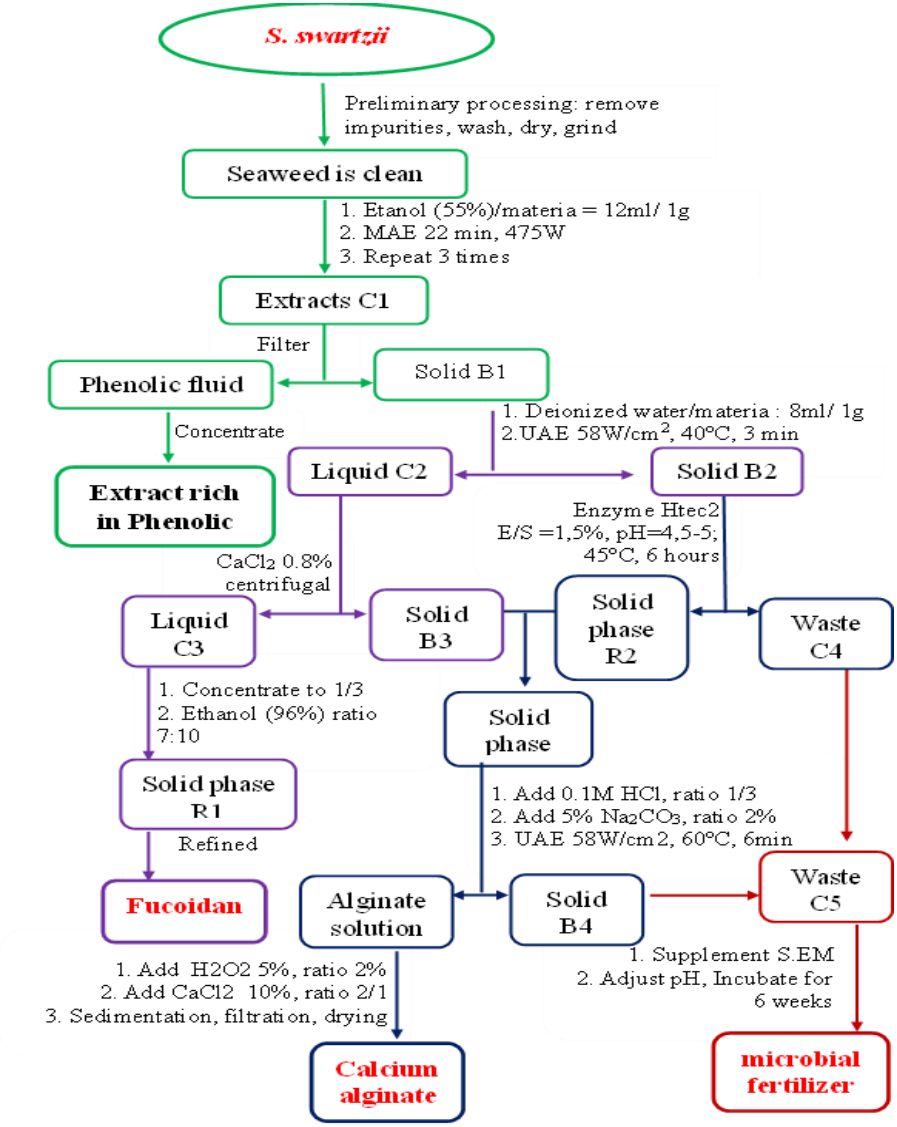

Figure 3.13. Comprehensive process diagram of seaweed processing

Process explanation

Step 1: Raw Material Preparation.

The harvested seaweed sample is cleaned, removing any foreign matter and organisms attached to it. It is rinsed first with seawater and then with fresh water. After drying, store it at a temperature of 5-10°C before use. When using, grind it finely using a specialized grinder.

Step 2: Phenolic Compound Extraction

Add 55% ethanol to the pre-processed seaweed (solvent-to-seaweed ratio: 12ml:1g). Perform extraction using a microwave oven at 475W power for 22 minutes, repeating the process three times. The resulting mixture (C1) is filtered to obtain phenolic solution and a solid residue mixture (B1). Concentrate the phenolic solution under reduced pressure to obtain a high-purity phenolic extract.

Step 3: Fucoidan Extraction

Mix the solid residue B1 with ion-deionized water (ratio: 10ml:1g). Apply ultrasonication at 58W/cm² and 40°C for 3 minutes. Filter the mixture after ultrasonication to obtain the extracted solution (C2) and solid residue (B2). Add 0.8% $CaCl_2$ to solution C2, stir for 1 hour, and then centrifuge to obtain a liquid phase (C3) and solid residue (B3). Concentrate the liquid phase C3 to 1/3 of its original volume. Add 96° ethanol (ratio to the concentrated solution: 7ml:10ml), let it stand for 1 hour, and then centrifuge at 10,000 rpm to obtain a solid phase (R1). Further purify to obtain clean fucoidan.

Step 4: Alginate Extraction, Bleaching, and Calcium Alginate Recovery

Solid waste B2 is hydrolyzed using the enzyme Htec2 at a concentration of 1.5% (enzyme/substrate). The pH is adjusted to approximately 4.5 - 5, and the temperature is maintained at 45°C for 6 hours. After hydrolysis, the mixture is cooled to room temperature, filtered, and centrifuged to obtain a solid phase (R2) and a waste solution (C4). The R2 solid phase is combined with solid waste B3, resulting in a mixed solid phase (R3). This mixture is treated with a 0.1 M HCl solution (at a ratio of 1/3) at 25°C. After filtration, the solid material is collected.

The collected solid material is washed with clean water and then supplemented with a 2% Na2CO3 solution. Ultrasonication is performed at a power of 58 W/cm2 and a temperature of 60°C for 6 minutes. The mixture is allowed to cool to room temperature, followed by filtration to obtain crude alginate solution and waste material B4. The crude alginate solution is gradually supplemented with a 5% H_2O_2 solution (at a ratio of 2%). It is stirred for 15 minutes and then slowly supplemented with a 10% $CaCl_2$ solution (at a ratio of 2/1). The mixture is allowed to settle for 2 hours, followed by filtration and drying to obtain the final product: Calcium alginate.

Step 5: Processing Residual Waste to Create Trace Element Organic Fertilizer. Combine waste solution C4 with waste residue B4 to create waste solution C5. Process waste solution C5 using the microbial product S.EM. Stabilize the pH. Allow the mixture to ferment for 6 weeks to obtain the final product: trace element organic fertilizer.

Table 3.10. Production efficiency at a scale of 5 kg of seaweed material/batch

Para-meter	Mass (kg)	Calcium Alginate Mass (g)	Alginate Content (%)	Calcium Content (%)	Fucoidan Mass (g)	High-Purity Phenolic Extract Mass (g)
1	5	212	80,1	5,8	14,5	8,50
2	5	209	81,2	6,2	15,1	8,81
3	5	209	80,23	5,7	15,0	8,49
4	5	214	81,5	5,5	14,8	8,53
5	5	210	80,9	5,7	15,2	8,55
6	5	213	81,3	6,1	15,5	8,48
7	5	212	81,5	6,0	14,9	8.52
8	5	215	81,7	6,3	15,3	8.50
9	5	218	82,0	6,2	14,8	8,54
10	5	220	80,6	5,9	15,2	8,53

Figure 3.13. Calcium Alginate Powder Product

3.7.2. Evaluate the effectiveness of different extraction processes and options

Table 3.12. Extraction Efficiency for Different Technical Approaches

Approach	Product Content (% based on dry weight of raw material)		
	Total phenolic	fucoidan	alginate
1: No UAE, no EAE, no MAE	0.015	1.23	8.24
2: EAE, no UAE, no MAE	0.088	1.97	16.6
3: UAE, MAE, no EAE	0.112	2.51	24.5
4: UAE, EAE, no MAE	0.182	2.78	28.7
5: MAE, EAE, UAE	**0.174**	**2.83**	**32.2**

Integrating Advanced Techniques Significantly Enhances Extraction Efficiency. Options 4 (ultrasonication and enzyme extraction) and 5 (microwave, ultrasonication, and enzyme integration) yield the best extraction efficiency.

Table 3.13. Comparison of Extraction Efficiency with Other Studies

Extraction Approach	Total Phenolic	Fucoidan	Alginate
Le Duc Giang et al (2016).	-	-	12.13-35.87%
Nguyen Van Nguyen (2018)	-	1.98-3.7%	-
Vo Mai Nhu Hieu (2014)	0.089 - 0.44%	-	-
Integrated Technology	**0.1704 – 0.182%**	**2.78 – 2.83%**	**28.7 – 32.2%**

Due to variations in the content and composition of Phenolic compounds, Fucoidan, and Alginate in seaweed, such as differences across species and seasons, it is challenging to accurately assess and compare extraction efficiency using different methods. However, the results from applying integrated advanced techniques demonstrate relatively high extraction efficiency. These approaches allow for the comprehensive extraction of valuable compounds from the raw material while utilizing waste byproducts for fertilizer, thereby providing economic benefits and environmental protection.

CONCLUSION AND RECOMMENDATIONS

1. The survey and evaluation of Brown seaweed samples collected from Vietnamese coastal areas revealed the following: **(i)** Sargassum seaweed has significant potential in terms of both quantity and quality for alginate isolation, with an average alginate content exceeding 30%; **(ii)** Lipid content in various Sargassum samples ranged from 0.07% to 2.11%, containing both saturated and unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs).

2. *In silico docking* screening identified that the compound difucodiphlorethol-A, isolated from Sargassum, has potential as a tyrosinase inhibitor for skin whitening product development.

3. The study evaluated the integration of advanced techniques (enzymes, ultrasonication, microwave, high-speed centrifugation) to establish comprehensive extraction and processing protocols for abundant Brown seaweed resources. This approach ensures synchronized recovery and high efficiency for valuable products such as phenolic compounds, fucoidan, and alginate. Additionally, utilizing waste byproducts as trace organic fertilizers contributes to minimizing environmental impact and enhancing the overall value of Brown seaweed.

4. Research Results and Process Development for Phenolic Extraction from Sargassum swartzii Using Microwave-Assisted Extraction and Response Surface Methodology (RSM): At an ethanol concentration of 55%, solvent/material ratio of 35/1 (v/w), extraction time of 65 minutes, and microwave output power of 475W, the obtained phlorotannin content was 1.50 ± 0.12 mg GAE/g, and the extract mass was 8.52 ± 0.11 mg. These values closely aligned with the predicted optimal conditions, as analyzed using Design Expert 7.0 software. In vitro antioxidant and cytotoxicity assays demonstrated moderate activity for the extracted phenolic compounds.

5. Integration of Advanced Techniques for Alginate Recovery and Calcium Alginate Production: Mini-pilot scale experiments were conducted to recover alginate and produce calcium alginate. Safety assessment revealed no toxicity at the tested dose. At concentrations of 20 µg/ml and 4 µg/ml, calcium alginate enhanced alkaline phosphatase (ALP) activity, collagen synthesis, and mineralization significantly compared to the negative control. Additionally, calcium alginate exhibited heavy metal chelation properties in mice, starting from a dose of 0.1 g/kg body weight.

Recommendations:

1. Continued Research and Product Diversification: Further research and explore the creation of additional valuable products from Brown seaweed: Extract phlorotannins for cosmetic applications; Extract trace minerals, vitamins, and lipids for functional food production.

2. Develop refined products using calcium alginate to detoxify heavy metals.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. Investigated and evaluated Brown seaweed samples collected from Vietnamese coastal areas; Identified suitable seaweed species for maximizing alginate extraction and calcium alginate production; Assessed the osteoporosis prevention activity, safety, and heavy metal chelation properties of calcium alginate.

2. Conducted virtual screening (in silico docking) of phenolic compounds from Brown seaweed to evaluate their potential as tyrosinase inhibitors; Provided guidance for further experimental studies in skin whitening product development.

3. Optimized the microwave-assisted extraction process for phenolic compounds from Brown seaweed using RSM.

4. Pioneered the integration of advanced techniques (ultrasonication, microwave, enzyme extraction) for comprehensive extraction of valuable compounds from Brown seaweed.