Free Radicals and Antioxidants, 2017; 7(1): 115-122
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.phcog.net

Original Article

Effects of extraction conditions over the phlorotannin content and antioxidant activity of extract from brown algae *Sargassum serratum* (Nguyen Huu Dai 2004)

Vu Ngoc Boi[1*], Dang Xuan Cuong[2], Phan Thi Khanh Vinh[1]**

[1]Nhatrang University, Ministry of Training and Education Nguyen DinhChieustreet, Nhatrang city, Khanhhoa province, VIETNAM.
[2]NhaTrang Institute of Technology Application and Researching, VAST Hungvuongstreet, Nhatrang city, Khanhhoa province, VIETNAM.

ABSTRACT

Introduction: This study focused on the discussion of effect of various extraction conditions for phlorotannin content and antioxidant activities extracted from brown algae *Sargassum serratum*. The algae was grown in the tropical coastal areas of Vietnam. **Methods:** The various extraction conditions include the following parameters: temperature (30-80^{0}C), maceration time (1/2–2,5 hours; 8-48 hrs), the ratio of solvent to material (10:1–70:1 (v/w)), pH (2–8), various kind of solvents (ethanol, acetone, chloroform, ethyl acetate and n-hexan) and solvent concentrations. Phlorotannin content, antioxidant activities, and some phytochemical compositions of *Sargassum serratum* were evaluated. **Results:** The highest phlorotannin content and antioxidant activities was expressed when extracting in the following conditions: 100% ethanol solvent at 50^{0}C in 32 hours with the ratio of solvent to material of 40/1 (v/w), pH 7 and one-step extraction. The extract contained flavonoid, terpenoid, alkaloid, fatty and oil. DPPH free radical scavenging of the extract was 87.53%. **Conclusion:** Phlorotannin content and antioxidant activities extracted from *Sargassum serratum* was depended on the extracting conditions. The condition of extraction for antioxidant phlorotannin and the chemical composition of extract was determined. Brown algae *Sargassum serratum* has high antioxidant phlorotannin content. A total of 6 compounds were identified from the extract of *Sargassum serratum*.

Key words: Antioxidant, Brown algae, Extraction, Phlorotannin, *Sargassum*.

Correspondence :

Vu Ngoc Boi,
Nhatrang University, Ministry of Training and Education Nguyen Dinh Chieu street, Nhatrang city, Khanhhoa province, VIETNAM.

E-mail: minhboint@yahoo.com
DOI: 10.5530/fra.2017.1.17

INTRODUCTION

Brown algae *Sargassum serratum* is one of the most potential *Sargassum* species, which were found along the coastal areas of Vietnam, especially in Nhatrang bay, Khanhhoa province. In years 2004, this algae was detected and classified in the first times in the world.[1]

Many studies showed that brown algae contained many biosubstances such as phlorotannin,[2,3] fucoidan,[4] alginate,[5]…. Inside, phlorotannins which have attracted considerable research interest because of their antioxidant activities.[6-9] Phlorotannins were formed from the basic units of phloroglucinol (1,3,5-trihydroxybenzene) and biosynthesized via polyketide pathway.[8-10] The molecular weights of phlorotannins were most found in the 10 to 100 kDa range.[2] Their content was ranged from 20-250 mg/g dry algae.[2,11]

In fact, phlorotannins content and bioactive activities was depended on brown algae species, site of growth algae, age of algae, harvesting methods and storage time.[2,12] Phlorotannin content and antioxidant activities which extracted from algae was depended on method of sample treatment as well as extraction conditions. The extraction conditions of phlorotannin and the bioactive activies from various brown algae species also inbited, species was grown in cold sea water such as *Fucus* sp.,[7] *Cystoseira*sp.,[13] *Eckloniastolonifera*,[14,15] *Fucusvesiculosus,*[16] and *Eiseniabicyclis*,[17] or in tropical sea such as: *Sargassum kjellmanianum.*[8-10]

Moreover, in recent years, there is an increased awareness and safety concern toward synthetic antioxidants and a worldwide trend to apply natural antioxidants to replace synthetic compounds as additives in foods or as functional food ingredients.[18,19] Natural antioxidants derived from various plants and marine algae not only show great potential for improving the oxidative stability of food products, but also have health-promoting benefits. Thus, some questions were happened such as phlorotannin exist or not exist in brown algae *Sargassum serratum,* how phlorotannin content, antioxidant activities of phlorotannin, polarization of phlorotannin, and extracting conditions effect on extracted phlorotannin and antioxidant activities is. These questions will be solved in the study. The results will contribute on the basic knowledge of brown algae phlorotannin, the technology of phlorotannin extract, and apply into health-promoting functional food formulations.

MATERIALS AND METHODS

Materials and chemicals

Algae materials: The brown algae *Sargassum serratum* Nguyen Huu Dai, 2004 was collected in the Nhatrang bay, on the south central coast of Vietnam in April, 2010. The algae were washed with clean seawater to remove epiphytes and sand attached to the surface and transported to the laboratory. The samples were carefully rinsed with tap water before drying using a heat-pump dryer. The final water content of dried algae was of 19 ± 1%. The dried samples were pulverized into powder and stored in polyethylene bags at 30$^{°C}$ prior to extraction.

Chemicals: Phloroglucinol, ascorbic acid, $FeSO_4$, Folin-Ciocalteu' sphenol reagent, $K_3[Fe(CN)_6]$, CCl_3COOH, NaH_2PO_4, Na_2HPO_4, ferric choride ($FeCl_3$), and ethanolwere purchased from Merck, Darmstadt, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ammonium Molybdate, sodium phosphate, Na_2CO_3, H_2SO_4 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Allother chemicals and reagents were of analytical grade.

Extraction of phlorotannins

The dried algae powder was extracted in various conditions such as various solvents (ethanol, acetone, chloroform, ethyl acetate and n-hexan); choiced solvent with different concentrations of 20, 40, 60, 80%, and

100%; the ratio of ethanol solvent to sample of 10:1, 20:1, 30:1, 40:1, 50:, 60:1 and 70:1 (v/w); different temperatures of 30, 40, 50, 60, 70 and 80°C; different times 0.5, 1.0, 1.5, 2.0, 2.5, 8.0, 16.0, 24.0, 32.0, 40.0 and 48.0 h; pH of 2, 3, 4, 5, 6, 7 and 8; and the repeat of extracting times in choiced condition. The extract were then centrifuged at 2500 rpm for 10 mins at 4°C and filtered with Whatman no. 4 filter paper, and analysed.

Determination of total phlorotannin content (PC)

The total phlorotannin content (PC) of extracts was quantified according to the Folin-Ciocalteu's method.[20] 300 µL of extracts was mixed with 1 mL of 10% Folin-Ciocalteu reagent. After keeping the mixture for 5 mins, 2 mL of 10% sodium carbonate was added. The samples were incubated for 1.5 h at room temperature in the dark. The absorbance was measured at 750 nm. Phloroglucinol was used as the standard. The results were expressed as milligrams of phloroglucinol equivalents (PGEs) per g dry weight of sample.

Total antioxidant activity

Total antioxidant activity (TA) of extracts was determined according to the method of Prieto *et al.* (1999).[21] 100 µL of extracts was mixed with 3 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and 900 µL of distilled water. The samples were incubated at 95°C for 90 min in water bath. The absorbance was measured at 695 nm. Ascorbic acid was used as the standard. The total antioxidant activity was expressed as mg ascorbic acid equivalents (AAEs) per dry weight of sample.

Reducing power

Reducing power (RP) was measured in following the method.[22] 500 µL of extract was added with 0.5 mL of 0.2 M phosphate buffer (pH 7.2) and 0.2 mL of 1% potassium ferricyanid ($K_3[Fe(CN)_6]$). After 20 min of incubation at 50°C, the mixture was added with 500 µL of 10% trichloroacetic acid (CCl_3COOH), 300 µL distilled water and 80 µL of 0.1% $FeCl_3$. The absorbance was measured at 655 nm. Ferrous sulfate was used as the standard, and the results are expressed as milligrams of ferrous sulfate equivalents (FSEs) per dry weight of sample.

DPPH radical scavenging

The DPPH radical scavenging activity was determined according to the method.[23] The sample solutions were prepared by adding 200, 400, 600, 800 and 1,000 µL of extract with 3 mL of DPPH solution (25 mg/L in methanol). The blank solutions (blank sample) was similar but DPPH was replaced by 3 mL of ethanol. The control samples was prepared similar to the blank sample but the extract was replaced by DPPH. The mixtures were votexed for 1 min and then left to stand for 30 min at 30°C in the dark. The absorbance was read at 550 nm. DPPH free radical scavenging activity was calculated according to the following equation:

$$A\% = \left[1 - \left(\frac{A_{sample} - A_{blank}}{A_{control}}\right)\right] \times 100$$

Where: A_{sample}: the absorbance of the sample solutions at 550 nm

$A_{control}$: the absorbance of the control samples at 550 nm

A_{blank}: the absorbance of the blank solutions at 550 nm

The absorbance was measured by using UV-vis spectrophotometer Jen Way 6400/6405.

Phytochemical determinations

The fat, oil, terpenoid, flavonoid and alkaloid contents were determined follow previous study.[24]

Statistical analysis

Each experiment was triplicated. Unusual data was removed by using Duncan method. Data was also analysed ANOVA and regression.

RESULTS

Effect of various solvents

Solvents with various polarization such as ethanol, acetone, ethyl acetat, chloroform and n-hexan were used while other parameters were unchanged. The Figure 1 indicated that phlorotannin content (PC) and antioxidant activities (AA) of extract was significantly influenced by the polarization of solvent ($p<0.05$). PC, TA and RP got the highest value of 4.102 ± 0.005 mg phloroglucinol equivalent/g dry weigh (DW),4.277 ± 0.0013 mg ascorbicacid equivalent/g DW and 24.228 ± 0.014 mg $FeSO_4$ equivalent/g DW, respectively, were shown in the Figure 1. There are a closely correlation between PC and AA ($R^2>0.9$). PC and AA was ranged in order to the increase of extracting solvent polarization as follow: n-hexan/ chloroform/ ethyl acetate/ acetone/ ethanol.

Effect of solvent concentration

The various concentrations of ethanol solvent were used and the other parameters were unchanged. PC and AA was significantly affected by concentration of solvent ($p<0.05$). 100% ethanol gave higher PC and AA of extract than using 20%, 40%, 60%, and 80% ethanol, respectively. PC, TA and RP were corresponded with the lowest value of 1.793 ± 0.001 mg phloroglucinol equivalent/g DW, 1.772 ± 0.002 mg ascorbic acid equivalent/g DW, and 10.438 ± 0.011 mg $FeSO_4$ equivalent/g DW when 20% ethanol was used, that things were shown in the Figure 2.

Effect of the solvent-to-material ratio

The Figure 3 showed the various ratios of solvent-to-material were used and the other parameters was unchanged. PC and AA was significantly impacted by the various solvent-to-material ratios ($p<0.05$). The correlation between the ratio of solvent-to-material and antioxidant PC was strong ($R^2>0.9$). The PC and AA was changed due to the regression models of level 2 when the ratio of solvent-to-material was increased from 10/1 (v/w) to 70/1 (v/w). The maximum point of the models of non linear was in the solvent-to-material of 40/1 (v/w). PC and AA was decreased and tend parallel to the horizontal axis when the ratio of solvent-to-material was greater 40/1 (v/w). It was reported in the Figure 3.

Effect of extracting temperature

Extract temperature was studied from 30°C to 80°C. The other parameters were unchanged. The results were presented in Figure 4. PC and AA was significantly impacted by the extracting temperature ($p<0.05$). PC and AA got the highest value and lowest value for the extracting temperature of 50°C and 80°C, respectively. PC, TA and RP got the maximum value of 4.532 ± 0.0051 mg phlorotannin equivalent/g DW, 5.375 ± 0.001 mg ascorbicacid equivalent/g DW and 26.757 ± 0.007 mg $FeSO_4$ equivalent/g DW, respectively for the maximum point. PC and AA was changed according to the model of level 2. A strong correlation between PC and TA, and RP was happened (R^2= 0.98 and 0.99, respectively), was showed in the Figure 4.

Effect of extracting time

The extracting time was surveyed from 0.5–2.5 hrs, 8 hrs-48 hrs. The other parameters were unchanged. The Figure 5 showed that PC and AA was significantly influenced by the extracting time ($p<0.05$). PC and AA was increased and decreased according to the model of non-linear regression with the increase of extracting time. The maximum point of model was happened with the extracting time of 32 hrs. The Figure 5 also showed the maximum value of PC, TA and RP got 4.569 ± 0.0057 mg phloroglucinol equvivalent/g DW, 5.376 ± 0.0033 mg ascorbic acid equvivalent/g DW and 26.754 ± 0.026 mg $FeSO_4$ equivalent/g DW, respectively. A strong correlation between PC and AA was happened (R^2> 0.97).

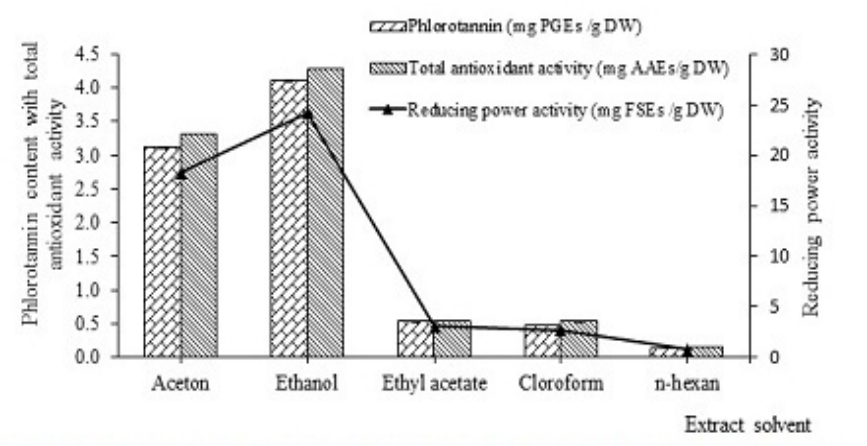

Figure 1: Phlorotannin content and antioxidant activities of various extracting solvents.

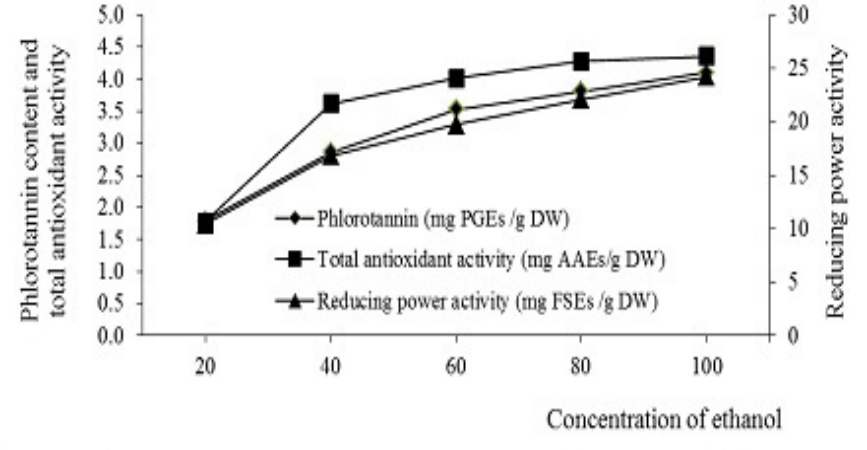

Figure 2: Phlorotannin content and antioxidant activities of various ethanol extract.

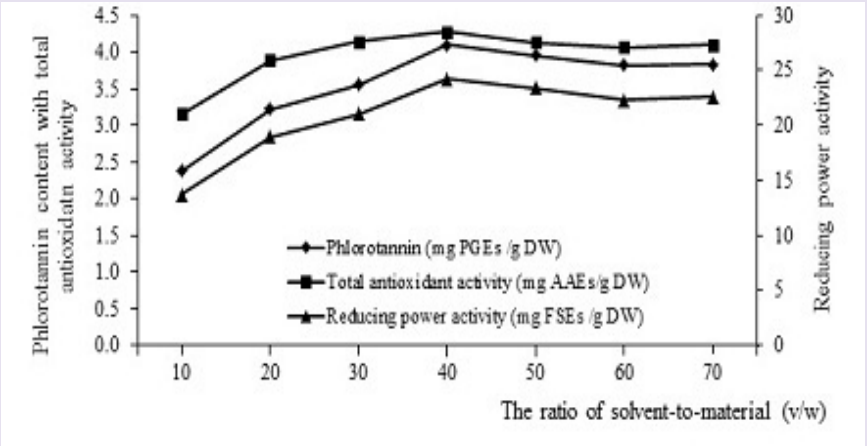

Figure 3: Effect of the ratio of solvent-to-material on extracted phlorotannin contentand antioxidant activities.

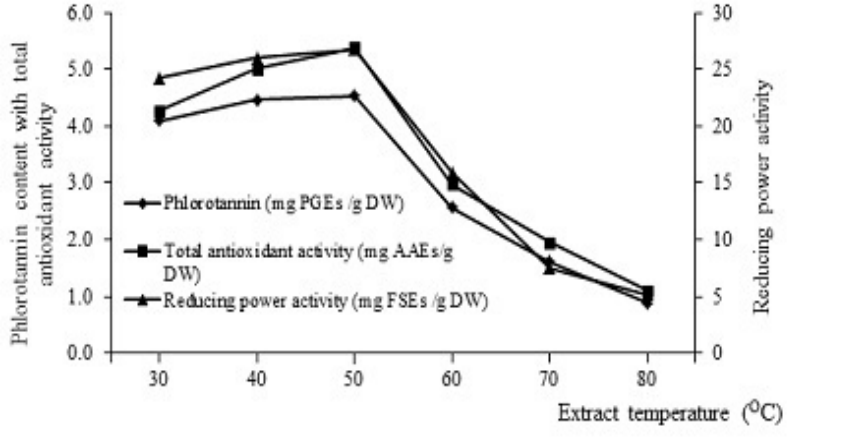

Figure 4: Effect of the temperature on extracted phlorotannin contentand antioxidant activities.

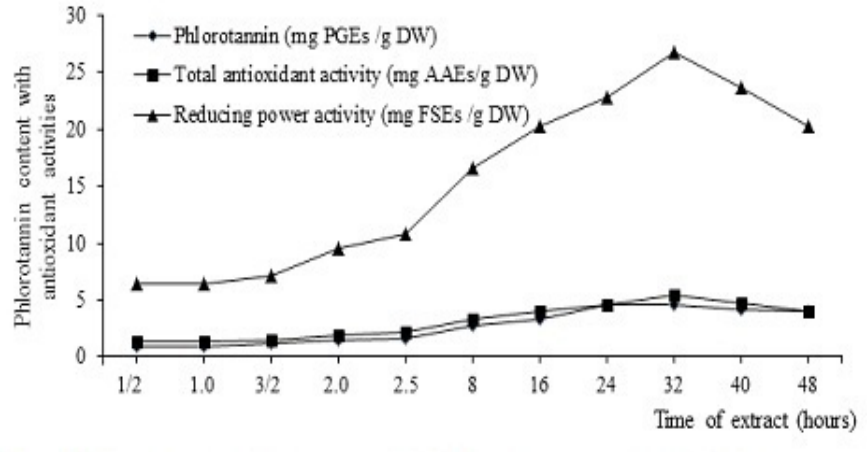

Figure 5: Effect of extracting time on extracted phlorotannin content and antioxidant activities.

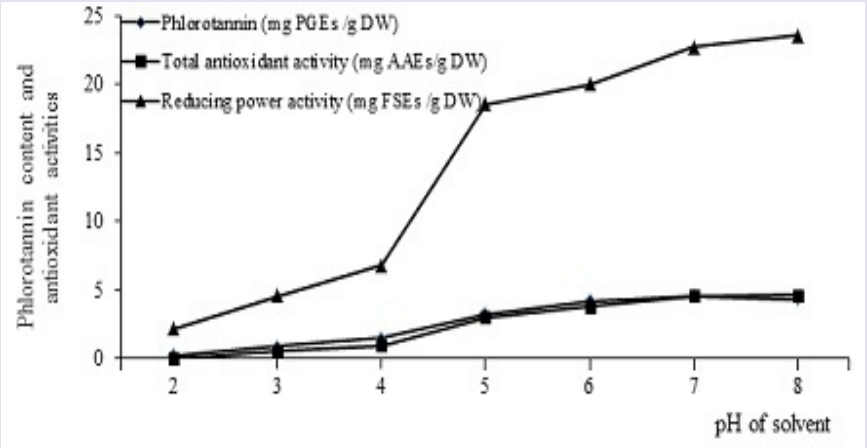

Figure 6: Effect of solvent pH on extracted phlorotannin content and antioxidant activities.

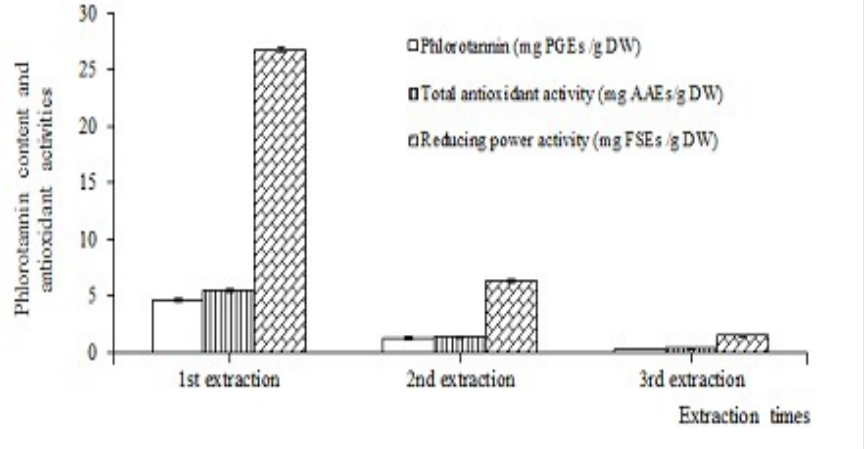

Figure 7: Effect of extraction times on extracted phlorotannin content and antioxidant activities.

Table 1: Some chemical composition qualitative of the extract of brown algae *S. seratum*

Compound group	Reagent/ performance method	Positive reaction	Result of qualitative	Result of general qualitative
Fatty	Drip extract on paper	Blur	++	Have
Oil	Evaporate to residue	Aromatic	+	Have
Terpenoid	Liebermann-Burchard	Brown red - violet, the green layer	+++	Have
Alkaloid	General reagent for alkaloid	Precipitation	+	Have
Flavonoid	Mg/HCl concentrate	Solution is pink to red	+	Have

Effect of pH of extract solven

pH of solvent was investigated from 2 to 8. The over chosen parameters were used for the study. The results showed that PC and AA got the maximum value at pH 7, was showed in the Figure 6. At pH 2, the acidity of solvent was the highest, PC and AA value were the lowest. PC was decreased by 94.24%, TA and RP was decreased by 99.63% and 90.36%, respectively, compared to PC and AA of extract at pH 7. The increasing of PC, TA, and RP got 0.39–1.7 mg phloroglucinol equivalent /g DW, 0.37–2.08 mg ascorbicacid equivalent/g DW and 1.46–11.76 mg $FeSO_4$ equivalent/g DW, respectively, when pH of solvent was changed from 2 to 7, it was reported in the Figure 6. At pH 8, PC was decreased to 0.233 mg phloroglucinol equivalent/g DW, TA, and RP was increased to 0.092 mg ascorbicacid equivalent/g DW, and 0.828 mg $FeSO_4$ equivalent/g DW, respectively, compared to PC and AA of extract at pH 7. The results also noticed that the strongly correlation between pH and AA was happened (R>0.9), and the closely causal relationship between PC and TA was also identified (R>0.9).

Effect of extract times

The Figure 7 showed that the closely relation between PC and AA was happened (R>0.9). For the extraction of 2nd times, PC, TA and RP only got 25.72%, 28.12% and 27.5%, respectively, compared to that of 1st extraction times. Continuously, for the 3rd extraction times, PC, TA and RP was obtained very little, they only got 0.272 ± 0.001 mg phloroglucinol equivalent/g DW, 6.23 ± 0.0014 mg ascorbic acid equivalent/g DW and 6.22 ± 0.001 mg $FeSO_4$ equivalent/g DW. The analysis shows that for the 1st extraction times, PC, TA and RP got 75.95%, 74.4% and 74.77%, respectively, compared to all 3 times of extraction.

Evaluation of phytochemical composition and DPPH free radical scavenging

The results of qualitative for the chemical compositions of extract were noticed in Table 1 and free radical scavenging DPPH was also presented in argumentation. Fatty, oil, terpenoid, alkaloid, flavonoid existed in the extract. Evaluation of free radical scavenging DPPH of extraction shows that DPPH activity got 87.53%, it was reported in the Table 1.

DISCUSSION

Phlorotannin content and antioxidant activities of phlorotannin was effected by various extracting conditions. The extract of brown algae *Sargassum serratum* contained 6 compounds. Antioxidant phlorotannin originated from brown algae *Sargassum serratum* was the diversity of structure and they were strongly effected by studied input factors. The Figure 1 appeared that non-polarization and polarization phlorotannin also exist in brown algae *Sargassum serratum*. Extracted phlorotannin have a diversity of structure and bioactives. Phlorotannin of polarization was more than one of non-polarization. The Figure 1 also showed that AA of non-polar extract was lower than that of polar extract. AA of ethanol extract was the highest. The antioxidant activities of non-polarization phlorotannin were lower than one of polarization phlorotannin. PC and AA was increased with the increase of solvent polarization. PC, TA and RP got the highest value and the lowest value when ethanol and acetone were used for extraction, respectively. The strong correlation expressed that PC had antioxidant activity. The previous study also performed in which polyphenol content in extract extracted from 6 brown algae *Sargassum filipendula, S. dublicatum, S. crassifolium, S. binderii* and

Padina sp. increased with the increase of solvent polarization and the ethanol solvent is the most sufficiency. However, antioxidant activity in other extract of different algae species was increased with the decrease of solvent polarization.[25] This is in accordance with the study of Takuo *et al.* (2011) that structure and biactivities of phlorotannin was diverse in plant.[26] Thus, phlorotannin originated from *S. serratum* was structure diversity and polarization. Moreover, ethanol is non-toxic solvent, and ethanol was also used for the extraction of polyphenol.[27] Thus, the ethanol solvent was sufficients to reach the high extraction, and it was used in the following experiments. The Figure 2 showed that the PC and AA was decreased with the decrease of solvent concentration. According to Quy *et al*. (2014), the PC and AA of the 100% ethanol extract was higher than that of other concentration extract.[28] A possible explanation for such a difference is that various gradients of ethanol concentrations,[29] extracellular and intracellular pressure of algae cell,[2] structure of algae cell, the structure characteristic of phlorotannin.[29] On the other hand, water impede the processing of penetration of solvent into the material. At the same time, water causes the algae cell to swell and a part of strong polarization phlorotannin was extracted by water. Water can not extract non-polarization phlorotannin. However, ethanol solvent can extract a part of non-polarization and polarization phlorotannin. Ethanol degrade cytoplasm of algae cell, penetrate deep into cell and help to increase the diffusion ability of solute to external environment. So ethanol concentration in solvent is higher, phlorotannin content is also higher. According to the description of Dongxiao *et al*. (2009), 100% ethanol was also used to evaluate the extraction efficiency for polyphenol extracts from kiwifruit.[30] Thus, ethanol 100% was best efficiency for extraction of phlorotannin and AA from brown algae *Sargassum serratum,* and chosen afterwards. Antioxidant phlorotannin content was extracted to due to the balance of solute between the solvent and intracellular of algae.[31] In addition, extracted phlorotannins were existed in free status, because the linkages between phlorotannins and the cell wall were destroyed. The ratio of solvent-to-material is greater, extracted phlorotannins are more and free phlorotannins are more. However, free phlorotannins were strongly impacted by the condition of extraction and ealier destroyed. Thus, destroyed phlorotannin are more than non-destroyed phlorotannin when the ratio of solvent-to-material was greater 40/1 (v/w). Other materials will have various conditions for extraction and the ratio of solvent-to-material was collected due to the purpose of study.[8,14,16] Results and interpretation are consistent with the theory of mass transfer was announced by Chan *et al*., (2014).[31] In brief, the ratio of solvent-material effect on extracted PC and AA from brown algae *Sargassum serratum.* The solvent-to-material ratio of 40/1 (v/w) was collected for afterwards, was showed in the Figure 3. Degradation of cell membrane and dissolving speed of phlorotannin was increased with the increase of extracting temperture. PC and TA was littler decreased, compared to the decrease of RP, when the extracting temperature was increased over 50^0C, was showed in the Figure 4. However, denaturation speed and decay of algae cell membrane was larger with the increase of extracting temperature. This is due to coagulation and change structure of cell,[31] simultaneously, degradation of phlorotannin. The results of Figure 2 showed phlorotannin of brown algae *Sargassum serratum* was not stable when the extracting temperatures was higher than 50^0C. The extracting temperature was due to various factors as: algae species, extract time, the ratio of solvent-to-material, size of sample.[3,9,16] Moreover, Pinelo *et al.* (2005) noticed that the temperature of 50^0C was used for extract polyphenol.[32] Thus, the temperature impact on PC and AA, and the temperature of 50^0C was collected for the extraction of phlorotannin and antioxidant activities in the follow study. The influence of the extracting time on antioxidant phlorotannin was published by the previous studies.[3,8,13,33,34] This thing shows the degradation of extracted phlorotannins was more and the stable of extracted phlorotannin was weaker when the extracting time was larger than 32 hrs, compared to extracted phlorotannin in 32 hrs, was showed in the Figure 5. The interactive between PC and TA (R^2 = 0.9771) was stronger than the interactive between PC and RP (R^2 = 0.9768). The time of extraction of phlorotannin and antioxidant activities was different for various materials.[3,8,13,33,34] According to Fick's law and the publish of Chan *et al*. (2014) shows that the solute was limited and got the maximum value in the special time.[31] Therefore, the extracting time effect on PC and AA, and the extracting time of 32 hrs was fit for the extraction of phlorotannin and antioxidant activities from brown algae *Sargassum serratum*. The Figure 6 showed that phlorotannin of brown algae *Sargassum serratum* was unstable in the acid and alkaline medium. In addition, acid and alkaline phlorotannin was less than neutral phlorotannin. PC and AA was increased with the increase of solvent pH. When pH of solvent was larger than 7, PC of extract was decreased, TA and RP of extract was increased, compared to that of extract at pH 7. According to the study of Rong (2010) shows that phlorotannin was extracted more by neutral medium.[35] Simultaneously, neutral pH of solvent was almost used for the extraction of polyphenol and bioactives.[34,36] Thus, pH of solvent also effect on PC and AA, and pH 7 was chosen for the extraction of antioxidant phlorotannin from brown algae *Sargassum serratum.* The Figure 7 showed that for the 3rd extraction times, PC and AA only got about 4.5%, compared to that of all 3 extraction times. PC, TA and RP of 3 times of extraction was not increased many, compared to that of 1st extraction times. After 3 times of extraction, PC and AA had a tend asymptotically horizontal axis. When solvent was used many, a processing of concentration will be difficults and solvent waste was much. Thus it is not necessary to extract many times. The impact, the extraction of one times was usually used for the studies of antioxidant activities and structure characteristic of phlorotannin in brown algae.[14,15,20,36] The extraction of many times was used for the determination of extraction yield, example: the extraction yield of phlorotannin was published.[8] The experiments of study shows the extraction of one times fit to the extraction yield, economic effective, as well as study time. Therefore, the extracting times also impact on extract PC and AA. The Table 1 noticed that flavonoid, terpenoid, alkaloid, fatty and oil existed in the extract of brown algae *Sargassum serratum*, inside terpenoid was more than various qualitated chemical compositions. However, terpenoid content can not equal or higher phlorotannin content. The previous studies showed bioactive compounds such as flavonoid, terpenoid, alkaloid, fatty and oil were common compounds in high level plant and low level plant.[37,38] Phytochemical composition of *S. serratum* extract was similar to that of *Erythrina indica* Lam. (Febaceae) leaves extract.[39-41] DPPH activity of phlorotannin extract was higher than DPPH activity of seagrasses leaf *C. rotundata* (78.84 ± 0.87%),[42] and that of leaf essential oil of *Syzygium lanceolatum* (69.97%).[43] However, structure, namely and content of concrete bioactive elements classified in these biocompounds was different for various plants.[10,44] Thus, new research directions and further researchs are need for these biocompounds. Algae waste was continuously used for the extraction of fucoidan after phlorotannin was extracted.[4] Alginate was continuously extracted after extracted fucoidan.[4] Finally residue was applied into fertilizer or biofuel. The phytochemical compositions of brown algae *Sargassum serratum* and other plants was homologously. The results of study will contribute in basic knowledge of phlorotannin of brown algae *Sargassum serratum* as well as the extracting technology of them.

In summary, phlorotannin extracted from brown algae *Sargassum serratum* had antioxidant activity and diversity of structure. Various extracting conditions effected strong on antioxidant phlorotannin, and antioxidant activity of extract generated from brown algae *Sargassum serratum.* In brown algae, there were many other compounds such as flavonoid, terpenoid, alkaloid, fatty and oil. When the effect and correlation of various extracting conditions on antioxidant phlorotannin,

and the correlation between phlorotannin and antioxidant activities was identified, the technology of antioxidant phlorotannin extraction can be fully controled for the collection of high antioxidant phlorotannin. In the studied method, the blemish of method was the long time and large solvent-to-material ratio for the extraction of antioxidant phlorotannin. However, the blemish did not effect on the accuracy of results. The method of microwave-assisted maceration and mix should be used to alternate the studied method. The long time and large S/M can be decreased when the replaced method was used. These things showed that the extract from brown algae *Sargassum serratum* was a resource of active elements. They can be fully applied into the field of functional food, foods and beverages, and medicine.

CONCLUSION

In this study, PC and AA of brown algae extract *Sargassum serratum* was strongly influenced by the input factors of various extracting conditions. Phlorotannin originated from *Sarrgassum serratum* had antioxidant activity and diversity structure. Moreover, flavonoid, terpenoid, alkaloid, fatty and oil was also contained in *Sargassum serratum*. Phlorotannin extract of antioxidant from brown algae *Sargassum serratum* can be used in foods, pharmacerticals and cosmetics. Besides, further studies of phlorotannin active ingredients as well as other bioactive compounds origined from brown algae *Sargassum serratum* should be investigated to apply widely in daily life. After phlorotannin extracting, algae waste should be used for the extraction of fucoidan, alginate and then produce fertilizer or biofuel.

ACKNOWLEDGMENTS

We are grateful to the staff of Nhatrang Institute of Technology Application and Research for collection of brown algae samples and Vietnam Academy of Science and Technology for money investment to research.

CONFLICT OF INTEREST

Authors have no conflict of interest.

ABBREVIATION USED

DPPH: 2, 2-diphenylpicrylhydrazyl; **PC:** The total phlorotannin content; **PGEs:** Phloroglucinol equivalents; **TA:** Total antioxidant activity; **AAEs:** Ascorbic acid equivalents; **RP:** Reducing power; **$K_3[Fe(CN)_6]$:** Potassium ferricyanid; **$FeCl_3$:** Ferrous triclorua; **FSEs:** Ferrous sulfate equivalents; **CCl_3COOH:** Trichloroacetic acid.

REFERENCES

1. Nguyen HD. Two new species of the subgenus Sargassum (Fucales, Sargassaceae) from Vietnam. In: Taxonomy of Economic Seaweeds with reference to the Pacific and other locations Volume IX. (Abbott, I.A. & Mc Dermid, K.J. Eds). 2004;9:73-80.
2. Koivikko R. Brown algal phlorotannins improving and applying chemical methods [Dissertation]. University of Turku, Finland. 2008.
3. Kuda T, Tsunekawa M, Hishi T, Araki Y. Antioxidant properties of dried 'kayamo-nori', a brown alga Scytosiphon lomentaria (Scytosiphonales, Phaeophyceae). Food Chemistry. 2005;89(4):617-22.
4. Elena MB, Sandra R, Andrés M, Herminia D, Juan CP. Simultaneous extraction and depolymerization of fucoidan from *Sargassum muticum* in aqueous media. Marine Drugs. 2013;11(11):4612-27.
5. Kokilam G, Vasuki S, Suja M. Bioactive potentials of brown seaweeds, *Sargassum myriocystum* J. Agardh *S. plagiophyllum* C. Agardh and *S. ilicifolium* (Turner) J. Agardh. International Research Journal of Pharmaceutical and Applied Science. 2013;3(5):105-11.
6. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Natural Products Report. 2012;29(2):144-222.
7. Jiménez-Escrig A, Jiménez-Jiménez I, Pulido R, Saura-Calixto F. Antioxidant activity of fresh and processed edible seaweeds. Journal of the Science of Food and Agriculture. 2001;81(5):530-34.
8. Yan X, Li X, Fan X, Zhou C. Studies on extraction procedure and antioxidative activity of phlorotannins from *Sargassum kjellmanianum*. Chinese Journal of Oceanology Limnology. 1997;15(1):42-5.
9. Wei Y, Xu Z. Studies on antioxidative activity of high molecular weight polyphenols from two kinds of brown algae. Zhongcaoyao. 2003;34(4):3170-319.
10. Yan X, Li X, Zhou C, Fan X. Prevention of fish oil rancidity by phlorotannins from *Sargassum kellmanianum*. Journal of Applied Phycology. 1996;8(3):201-3.
11. Ragan MA, Glombitza KW. Phlorotannins, Brown algal polyphenols. Progress in Phycological Research. 1986;4:129-241.
12. Dang XC, Vu NB, Tran TTV, Le NH. Effect of storage time on phlorotannin content and antioxidant activity of six Sargassum species from Nhatrang bay, Vietnam. Journal of Applied Phycology. 2015;28(1):567-72.
13. Chkhikvishvili ID, Ranazanov ZM. Phenolic substances of brown algae and their antioxidant activity. Applied Biochemitry and Microbiology. 2000;36(3):289-91.
14. Kang HS, Chung HY, Jung JH, Son BW, Choi JS. A new phlorotannins from the brown alga *Ecklonia stolonifera*. Chemical and Pharmaceutical Bulletin. 2003;51(8):1012-4.
15. Kang HS, Chung HY, Kim JY, Son BW, Jung HA, Choi JS. Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. Archives of Pharmacal Research. 2004;27(2):194-8.
16. Koivikko R, Loponen J, Pihlaja K, Jormalainen V. High-performance liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus*. Phytochemical Analysis. 2007;18(4):326-32.
17. Takashi N, Kohki N, Kenji U, Ryusuke T. Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicyclis*. Fisheries Science. 1996;62(6):923-6.
18. Balasundram N, Sundram K, Samman S. Phenolic compounds in plant and agri-industrial byproducts: antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006;99(1):191-203.
19. Kanti BP, Syed IR. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity. 2009;2(5):270-8.
20. Swanson AK, Druehl LD. Induction, exudation and the UV protective role of kelp phlorotannins. Aquatic Botany. 2002;73(3):241-53.
21. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry. 1999; 269(2):337-41.
22. Zhu QT, Hackman RM, Ensunsa JL, Holt RR, Keen CL. Antioxidative activities of oolong tea. Journal of Agricultural and Food Chemistry. 2002;50(23):6929-34.
23. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-200.
24. Soni H, Sharma S, Patel SS, Mishra K, Singhai AK. Preliminary phytochemical screening and HPLC analysis of flavonoid from methanolic extract of leaves of *Annova squamosal*. International Research Journal of Pharmacy. 2011;2(5):242-6.
25. Bambang BS, Kumalaningsih S, Susinggih W, Hardoko. Polyphenol content and antioxidant activities of crude extract from brown algae by various solvents. Journal of Life Science and Biomedicine. 2013;36:439-43.
26. Takuo O, Hideyuki I. Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. Molecules. 2011;16(3):2191-217.
27. SineiroJ, Domínguez H, Núñez MJ, Lema JM. Ethanol extraction of polyphenols in an immersion extractor. Effect of pulsing flow. Journal of the American Oil Chemists' Society. 1996;73(9):1121-5.
28. Quy DD, Artik EA, Phuong LTN, Lien HH, Felycia ES, Suryadi I. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophilaaromatica. Journal of Food and Drug Analysis. 2014;2(3):296-302.
29. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Foline-Ciocalteu methods. Food Chemistry. 2006;99(4):835-41.
30. Dongxiao SW, Ivy W, Reginald W, Laurence DM, Sandhya W. Evaluation of the extraction efficiency for polyphenol extracts from by-products of green kiwi fruit juicing. International Journal of Food Science & Technology. 2009;44(12):2644-52.
31. Chan CH, Yusoff R, Ngoh GC. Modeling and kinetics study of conventional and assisted batch solvent extraction. Chemical Engineering Research and Design. 2014;92(6):1169-86.
32. Pinelo M, Rubilar M, Jerez M, Sineiro J, Núñez MJ. Effect of solvent, temperature and solvent-to-solid ratio on the total phenolic content and antiradicalary activity of extracts from different components of grape pomace. Journal of Agricultural Food Chemistry. 2005;53(6):2111-7.
33. Mayalen Z, Daniel R, Yolanda FP. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. Journal of Applied Phycology. 2007;19(5):449-58.
34. Yangthong M, Hutadilok-Towatana N, Phromkunthong W. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Foods for Human Nutrition. 2009;64(3):218-223.
35. Rong T. Chemistry and Biochemistry of Dietary Polyphenols. Nutrients. 2010;2(12):1231-46.

36. Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT Food Science Technogy. 2008;41(6):1067-72.
37. Nishanthi R, Karpanai SB, Sobana PP, Logeswari V, Kathiresan E, Tamilselvi A. Phytochemicals, antimicrobial and antioxidant screening from five different marine microalgae. Journal of Chemical and Pharmaceutical Sciences. 2014;2 (Special Issue):78-85.
38. Xiuzhen H, Tao S, Hongxiang L. Dietary polyphenols and their biological significance. International Journal of Molecular Sciences. 2007;8(9):950-88.
39. Hong-Yu L, Bin W, Chun-Guang Y, You-le Q, Chuan-ling S. Evaluation of antioxidant activities of five selected brown seaweeds from China. Journal of Medicinal Plants Research. 2010;4(18):2557-65.
40. Rastian Z, Mehranian M, Vahabzadeh F, Sartavi K. Antioxidant activity of extract from a brown alga. *Sargassum boveanum*. African Journal of Biotechnology. 2007;6(24):2740-5.
41. Sakat SS, Juvekar AR. Comparative study of Erythrina indica Lam. (Febaceae) leaves extracts for antioxidant activity. Journal of Young Pharmacists. 2010; 2(1):63-7.
42. Danaraj J, Ponnnambalam S, Subramaniyan R, Karmegam A, Thirunavukarassu T. Evaluation of *in-vitro* antioxidant activity of seagrasses: signals for potential alternate source. Free Radicals and Antioxidants. 2016;6(1):77-89.
43. Chellam M, Nadarajan S, Arun KD, Pujari SS. Chemical profiling of leaf essential oil, antioxidant potential and antibacterial activity of *Syzygium lanceolatum* (Lam.) Wt. & Arn. (Myrtaceae). Free Radicals and Antioxidants. 2016;6(1):13-22.
44. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011;3(12):10-14.

SUMMARY

- The effect of extracting condition on antioxidant phlorotannin content of *Sargassum serratum* extract was studied. The result showed, antioxidant phlorotannin content was effected by various extracting conditions, and flavonoid, terpenoid, alkaloid, fatty and oil was existed in the extract.

ABOUT AUTHOR

Dr. Vu Ngoc Boi: Has completed B. Biology (speciality in Biochemistry) from Vietnam National University - Hanoi University of Science (VNU University of Science). He obtained his PhD Degree in Biochemistry from University of Science, Vietnam National University-Ho Chi Minh City. Currently he is working as a Dean of Food Technology Faculty, Nha Trang University, Vietnam. Dr. Vu Ngoc Boi has carried out extensive research in the area of enzymatic hydrolysis of fish flesh and enzyme-assisted extraction of carrageenan from Kappaphycus alvarezii Doty (Doty), extraction of fucoidan, phlorotannin from Sargassum. He has guided 29 Masters and 08 Doctoral Scholars.