P-FNN-21

Phenolic and Flavonoid Contents Isolated from the Red Seaweed, *Acanthophora spicifera*

Abdulmuhaimin Bilanglod, Sittiporn Petthongkhao and Nunta Churngchow

Department of Biochemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

***** *Corresponding author. E-mail: nunta.e@psu.ac.th*
DOI :

ABSTRACT

Nowadays, seaweeds have been considered as sources of bioactive compounds used for antioxidant and anti-pathogen in medical experiments and cosmetic ingredients including used as elicitor for induced resistance in agricultural sciences. *Acanthophora spicifera* is a non-valuable red seaweed that can be found in Andaman sea and gulf of Thailand. In this study, we have used this red seaweed to search for its bioactive compounds that could be served as alternative sources. Two methods were compared between extracted with hot water and methanol. Using hot water, we obtained high content of phenolic; in contrast, using methanol gave high amount of flavonoid. The phenolic content using hot water was 3.42 mg gallic acid equivalent/g dry weight and flavonoid content extracted with methanol was 2.93 mg rutin equivalent/g DW. We measured *in vitro* antioxidant activities using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) methods. For DPPH, the IC_{50} of DPPH scavenging activity using hot water and methanol were 7.33 mg and 25.32 mg, respectively. For FRAP, the hot water extract had reducing activity higher than the methanol extract which were 8.08 and 4.66 mg ascorbic acid equivalent/g DW, respectively. In conclusion, the hot water extract had antioxidant activity higher than the methanol extract. These results provided the evident of using low cost extraction method but obtained higher amount of active compounds; consequently, could up value of this non-valuable seaweed. The ABTS Scavenging of (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) activity, anti-tyrosinase and antibacterial activities will be further conducted before applying these active compounds on food and cosmetic industries.

Keywords: Red seaweed, *Acanthophora spicifera*, Phenolic, Flavonoid, Antioxidant Activity

INTRODUCTION

Nowadays, the demand of antioxidant using for daily life is becoming more important because exposures to free radical by using drugs, by contacting with pollution and cigarette smoke are increased. Free radicals are unstable atoms or molecules which can oxidize neighboring molecules to make themselves more stable that is the result of free radical chain reaction. Free radicals are derived from both endogenous and exogenous sources. Endogenous sources include lipid oxidation and xanthine oxidase which can catalyse xanthine to uric acid and hydrogen peroxide radical. For exogenous sources; there are UV, X-ray, gamma-ray, ozone and cigarette smoke. Most of the free radicals are in the form of reactive oxygen species such as oxygen radical and superoxide radical. These reactive oxygen species can cause oxidative stress. They can damage tissues leading to many diseases such as Parkinson and Alzheimer diseases. Antioxidants are substances that can inhibit or repress oxidation slowly by giving hydrogen radical that make free radical become more stable. Our body also produces some antioxidants, which are known as endogenous antioxidants such as glutathione peroxidase. Antioxidants that come from outside the body are called exogenous including ascorbic acid, phenolic, flavonoid and carotenoid.

Seaweeds are considered to be a rich source of antioxidant (Cahyana, Shuto, & Kinoshita, 1992; Lim, Cheung, Ooi, & Ang, 2002). Different types of solvents have been used to extract antioxidants from various species of seaweeds (Yan, Chuda, Suzuki, & Nagata, 1999). For example, Aroa Lopes *et al.*, (2011) used water, water: methanol (1:1), methanol and ethanol to extract bioactive compounds from *Stypocaulon scoparium* alga. The water extract showed the highest antioxidant activity and highest phenolic content than other solvents. Massoumeh *et al.*, (2014) used methanol to extract phenolic and flavonoid from four *Ulva* species (*Ulva clathrata* (Roth) C. Agardh, *Ulva linza* Linnaeus, *Ulva flexuosa* Wulfen and *Ulva intestinalis* Linnaeus). They showed that methanolic extract had higher flavonoid than phenolic contents in all of four *Ulva* species. In our study, we aimed to analyze antioxidant activity of phenolic and flavonoid contents extracted from *Acanthophora spicifera* by comparing two extraction methods (hot water and methanolic extractions).

MATERIAL AND METHODS

Materials

Ascorbic acid, Folin-ciocalteu reagent, and methanol were purchased from Merck company. Gallic acid, DPPH, TPTZ (2,4,6-tripyridyl- striazine), and rutin were purchased from Sigma Chemical Co (St. Louis, MO, USA). All of chemicals and reagent used were of analytical grade.

Preparation of extracts

Hot water extract. was prepared by boiling 5 g red alga powder in 500 ml of distilled water at 80°C for 60 min. Then, filtered and centrifuged at 6,500 rpm for 20 min. The supernatant was lyophilized and stored at 4 °C until use.

Methanolic extract. was prepared by overnight mixing 5 g red alga powder in 500 ml of methanol at room temperature. Then, methanol solution was collected from remaining red alga residue by passing through Whatman filter paper *(*No. 4*)*. The solution was concentrated and made into dried powder by rotary evaporation and lyophilization techniques, respectively. The dried powder was kept at 4°C until use.

Determination of phenolic content

The phenolic content was determined by Folin- Ciocalteu method previously described by Moreira *et al.*, *(*2008*)*. Each extract solution *(*0.2 ml*)* was added to 0.5 ml of 1 N Folin-Ciocalteu reagent. After 5 min, 1 ml of 20% sodium carbonate was added. After incubation of mixtures at room temperature for 10 min, the absorbance at 730 nm was measured. Gallic acid *(*2.5-12.5 ug*)* was used for the standard calibration curve and the results were presented as gallic acid equivalent *(*GAE*)*/g DW of alga.

Determination of flavonoid content

Flavonoid content was determined by Aluminium chloride colorimetric method. Each sample *(*0.25ml*)* was diluted in 0.25 ml methanol and added of 1.5 ml ethanol, 2.8 ml distilled water, 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M potassium acetate. The mixture was incubated at room temperature for 30 min and analyzed by measurement of absorbance at 415 nm using rutin *(*5-25 ug*)* as standard. Flavonoid content was expressed as mg rutin equivalent *(*RE*)*/ g DWof alga powder

In vitro antioxidant activities

DPPH assay (2,2- diphenyl- 1- picrylhydrazyl). DPPH radical scavenging activity was determined by a modified method previously described by Wang *et al.*, (2012). Briefly, extract solutions (0.2 ml) was reacted with 1 ml of 0.1 mM DPPH solution in methanol. The mixture was stood at room temperature in the dark for 30 min. Absorbance was measured at 517 nm in order to calculate for IC50 (IC50 value was defined as an effective concentration for scavenge 50% radical). Trolox (6- Hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as positive control.

FRAP assay (ferric reducing antioxidant power). FRAP assay was analyzed by modified method previously described by Benzie and Strain, (1996). Prepared FRAP reagent (3 ml) containing 100 ml of 0.3 M acetate buffer pH 3.6, 10 ml of 10 mM TPTZ solution, and 10 ml of 20 mM ferric chloride solution was mixed with 0.10 ml of sample solutions and incubated at room temperature for 6 min. Absorbance was measured at 593 nm. L-ascorbic acid (2-10 ug) was used for the standard calibration curve. The results of reducing ability were presented as mg L-ascorbic acid equivalent (AAE)/g DW of alga powder.

RESULTS

Phenolic and Flavonoid Contents

The phenolic and flavonoid contents of hot water and methanolic extracts from red algae (*A. spicifera*) were determined and presented in term of mg of Gallic Acid Equivalents (mg GAE) and mg of Rutin Equivalents (mg RE), respectively (Figure 1). The hot water extract had higher phenolic content than the methanolic extract which were 3.42±22.40 and 1.02±22.40 mg gallic acid equivalent/g DW, respectively (Figure 1). In contrast, the methanolic extract had higher flavonoid content than the hot water extract which were 2.93±19.74 and 0.58±71.17 mg rutin equivalent/g DW, respectively (Figure 1). The results suggested that two extraction methods had different ability to extract phenolic and flavonoid from *A. spicifera*

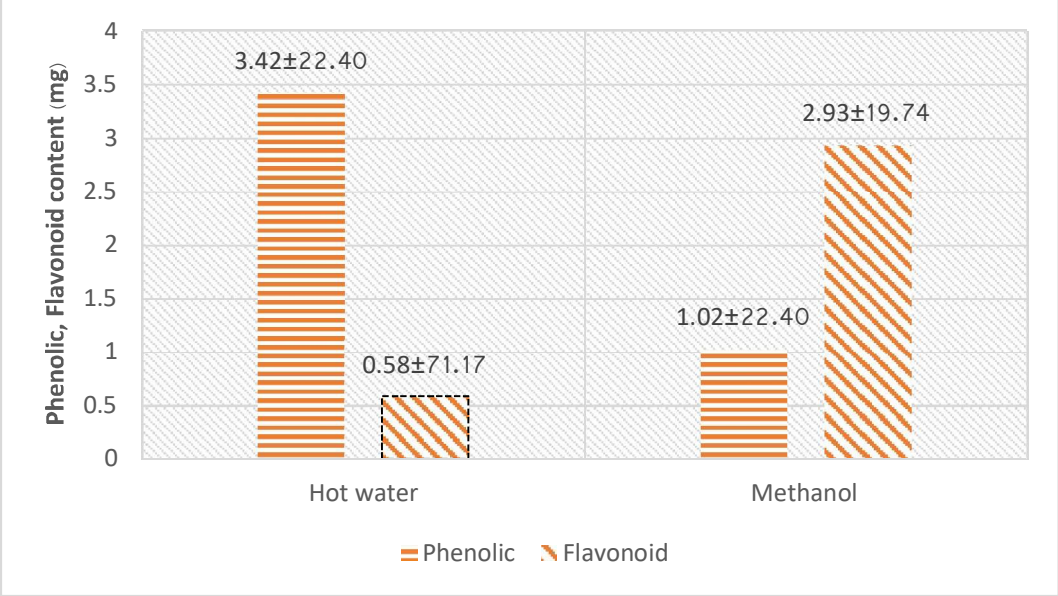

Figure 1. Phenolic and flavonoid contents presented as mg GAE and mg RE, respectively, of hot water and methanolic extracts from *A. spicifera*

DPPH radical scavenging activity

Obtained extracts were also used for determination of DPPH radical scavenging activity. Hot water extract showed higher DPPH scavenging activity than methanolic extract (Table 1). The IC_{50} values of hot water and methanolic extracts were 7.33±0.023 and 25.32±0.034 mg, respectively (Table 1). The result indicated that hot water did could extract higher DPPH scavenging activity compounds than methanol.

Table 1. IC_{50} of hot water and methanolic extracts in DPPH radical scavenging activity

Sample	IC_{50} of DPPH scavenging activity (mg)
Hot water	7.33 ±0.023
Methanol	25.32±0.034
L-ascorbic acid	0.0055±0.005

Ferric Reducing Antioxidant Power (FRAP)

FRAP is assayed based on ability of antioxidants that can reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ (Zengin *et al.*, 2015), resulting in forming of intense blue Fe^{2+} TPTZ complex which has maximum absorption at 593 nm. L-ascorbic acid (2-10 ug) was used for a standard calibration curve. The results of reducing ability were expressed as mg L-ascorbic acid equivalent (AAE)/g DW of alga powder. As shown in table 2, the FRAP value of the hot water extract (8.08± 0.25 mg AAE/g dry weight) was higher than that of methanolic extract (4.66± 0.14 mg AAE/g DW). The result indicated that hot water could did extract higher ferric reducing antioxidant compounds than methanol had.

Table 2. Ferric reducing antioxidant power ability of hot water and methanolic extract

Sample	Reducing ability (mg AAE/g DW)
Hot water	8.08± 0.25
Methanol	4.66± 0.14

DISCUSSION

The chemical extraction depends on the types of solvents with varying polarities, pH, extraction time and temperature as well as on the chemical composition of the sample (Aroa *et al.*, 2011). From our results two methods which are hot water and methanol extractions were compared. We found that hot water extract gave higher phenolic compounds than methanolic extract, in contrast, methanolic extract gave high amount of flavonoid. Aroa *et al*., (2011) also showed that after using solvent to extract phenolic from alga (*Stypocaulon scoparium*), water extract showed higher phenolic content than methanol extract. Hot water was the effective solvent for phenolic extraction which was similar to the finding reported by Xu *et al.*, (2007). They used hot water to extract phenolic compounds from citrus peel and detected that phenolic content was correlated with temperature used. Massoumeh *et al*., (2014) used methanol to extract and found that the major component of the extract was flavonoid. From our result, content of phenolic was related to the antioxidant activity of the extract rather than the content of flavonoid. It has been demonstrated that

phenolic compounds are one of the most effective antioxidants in marine algae (Zakaria et al., 2011)

CONCLUSION

The hot water and methanolic extracts from red alga *A. spicifera* gave high phenolic and flavonoid contents, respectively. The DPPH scavenging activity and ferric reducing power ability of hot water extract were higher than methanolic extract. These results provided the evident of using low cost extraction method but obtained higher amount of active compounds. Our study could be up value of this non-valuable seaweed. These active compounds could be further considered for applying on food and cosmetic industries.

ACKNOWLEDGEMENTS

This work was supported by the Academic Excellence Enhancing Program in Biochemistry, Prince of Songkla University. Research Grant for Thesis from Graduate School, Prince of Songkla University and Academic Presentation Expenses Support from Science Graduate Prince of Songkla University.

REFERENCES

Aro, L., Milagres, R., Argimiro, R., Miguel, S.T. 2011. The effects of solvents on the phenolic contents and antioxidant activity of *stypocaulon scoparium* algae extracts. Jounal of Food Chemistry, 125: 1104-1109.

Benzie, I.F.F. and strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power" the FRAP assay. Anal. Biochem. 239: 70-76

Cahyana. A.H., Shuto, Y. and Kinoshita, Y. 1992. Pyropheophytin a as an antioxidative substance from marine alga, Arame (*Eiseniabicyclis*). Bioscience, Biotechnology and Biochemistry, 56: 1533-1535.

Ganesan, P., Kumar, C.P., Bahaskar, N. 2008. Antioxidant properties of methanol extracts and its solvent fractions obtained from selected Indian red seaweed. Jounal of Bioresources Technology. 99:2717-2723.

Hua-Bin, L., Ka-Wang, C., Chi-Chun, W., King- Wai, F., Feng, C., Yue, Y. 2007. Evaluation of antioxidant capacity and total phenolic content of

different fraction of selected microalgae. Journal of Food Chemistry. 102: 771-776.

Lim, S.N., Cheung, P.C.K., Ooi, V.E.C., and Ang, P.O. 2002. Evaluation of antioxidative activity of extract from a brown seaweed, *Sargussum siliquastrum*. Journal of Agricultural Food Chemistry, 50: 3862-3866.

Liping, S., Yan, G., Yanxin, Z., and Yongliang, Z. 2017. Antioxidant and antityrosinase activities of phenolic extracts from rap bee pollen and inhibitory melanogenesis by cAMP/ MITF/ TYR pathway in B16 mouse melanoma cells. Front. Pharmacol. 8: 104.

Massoumeh, F., Ramazan-Ali, K.N., Seyed mohammad, B.N., Foroogh, N. 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweed from Northern Coasts of the Persian Gulf. Iranian journal of Pharmaceutical Research. 13 (1): 163-170.

Moreira, L., Dias, L.J., Percira, J.A. and Esterinho, L. 2008. Antioxidant properties, total phenol and pollen analysis of propolis sample from Portugal. Food Chem. Toxical. 46: 3482-3485.

Wang, B.N., Liu, H.F., Zheng, J.B., Fan, M.T., and Ca, O.W. 2011. Distribution of phenolic acids in different tissues of jujube and their antioxidant activity. J. Agric. Food Chem. 59: 1288-1292.

Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., Jiang, P. and Ye, X.Q., 2007. Mineral, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. Journal of Food Science. 73.

Yan, X.J., Chuda, Y., Suzuki, M., and Nagata, T., 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed, *Bioscience*, Biotechnology and Biochemistry, 63: 605-607.