International Food Research Journal 23(6): 2370-2373 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluation of heavy metal, antioxidant and anti-tyrosinase activities of red seaweed *(Eucheuma cottonii)*

Chang, V.S. and *Teo, S.S.

Department of Biotechnology, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Height, Cheras, 56000, Kuala Lumpur, Malaysia

Article history

Received: 7 September 2015
Received in revised form: 24 February 2016
Accepted: 7 March 2016

Keywords

Heavy metal
Antioxidant
Anti-tyrosinase
Red Seaweed
Eucheuma cottonii

Abstract

Seaweeds are marine macro algae that can be found attach to the bottom shallow coastal waters. There are three major groups of seaweeds namely brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta). One of the edible red seaweeds is *Eucheuma cottonii*. Red seaweeds have been found of consisted several potential pharmaceutical uses such as antitumor, antiviral, anticoagulant and immunomodulation functions. In This study, heavy metals content of *E. cottonii* was determined by ICP-OES. Methanol was used as solvent for extraction. The phenolic content of the extract was determined by Follin-Ciocalteau method and results were expressed as gallic acid equivalents. The antioxidant activity was determined by DPPH assay. Besides, anti-tyrosinase activity was investigated tyrosinase and L-DOPA with kojic acid as positive control. For element test, three elements were detected (Ar, Fe and Zn). The phenolic contents (3.40±0.013 mg GAE/g extract) and antioxidant activity (38.82±0.99 mg/mL) were lower compared to other plants but *E. cottonii* shows a good tyrosinase enzyme inhibition which achieved average 234.33µg/mL in 50% inhibition tyrosinase concentration (IC_{50}). *E. cottonii* could be the potential source of natural anti-tyrosinase

© All Rights Reserved

Introduction

Marine macroalgae, also commonly known as seaweeds are well known with their wealth of bioactive compounds particularly the polysaccharide and thus, provide great biological active resources. For example, compounds isolated from marine macroalgae have demonstrated various biological activities, such as antibacterial activity, antioxidant potential, anti-inflammatory properties, anti-coagulant activity, anti-viral activity and apoptotic activity (O'Sullivan *et al*., 2010). *Eucheuma cottonii* is an edible species of red seaweeds which mainly harvested in Malaysian North Borneo Sabah. It is a spiny bushy marine plant, about 50m in height, which grows on reefs and shallow lagoons. *E. cottonii* is a potential source of a variety of compounds like dietary fibers, vitamin C, α-tocopherol, minerals, fatty acid and protein (Matanjun *et al*., 2009; Jiménez *et al.*, 2010).

Antioxidant is a substance that able to delays or inhibits oxidation of the molecules by initiation of oxidizing chain reactions initiated by free radicals (Velioglu *et al*., 1998). Antioxidant can be divided into two groups, exogenous (eg: vitamin c, vitamin E and polyphenols) and endogenous antioxidants (eg: antioxidant enzymes and metal binding proteins). Tyrosinase is a copper-containing enzyme where it involved in formation of melanin pigments (Petit and Pierard, 2003; Kim and Uyama, 2005; Chang, 2009). Physiologically, skin naturally stimulating tyrosinase enzyme for melanin production to protect skin damaged by ultraviolet A (UVA) and ultraviolet B (UVB) in sunlight. Briefly, tyrosinase (EC 1. 12.18.1) or phenol oxidase is a melanogenesis copper-containing enzyme (Özer *et al*., 2007). Tyrosinase transforms L-tyrosine into L-DOPA by hydroxylation, and further into o-dopaquinone by oxidation and eventually melanin (Kamkaen *et al*., 2007). The accumulation of melanin lead to hyperpigmentation of skin darkening and will causes dermatological disorders such as age spots and freckling (Narayanaswamy *et al*., 2011). There are various causes for hyperpigmentation including increases of melanin and melanocytes and anti-tyrosinase molecules is critical to inhibit enzymatic activity of tyrosinase by chelating of copper atoms on the enzyme molecule (Özer *et al*., 2007). Without copper atoms, transformation of tyrosine to melanin will be slowing down and decreases skin hyperpigmentation as a result.

Samples rich with antioxidant always related with anti-tyrosinase, this is because of both antioxidant and anti-tyrosinase play important roles in preventing

*Corresponding author.
Email: *angeliaucsi@gmail.com*

free radical-related skin damage. The aim for this project is to investigate the elements contents of *E. cottonii*, to assess the antioxidant and anti-tyrosinase properties of *E. cottonii*.

Materials and Methods

Specimen of red seaweed was collected from the coastal areas of Sabah, Malaysia. *E. cottonii* was collected from Semporna (east coast of Sabah) water. Fresh seaweed was washed with fresh water and dry under the sun. The dried seaweed was then brought back to lab and wash with tape water. The cleaned seaweed was oven dry at 40ºC for 36 hours. The dried sample was pulverized with mechanical grinder and obtaining fine powder by passed through sieve.

Macro and trace elements

The seaweed was washed and dried at 40°C for 3 days. The seaweed sample was carried out by using AOAC official method 999.10 to determine the heavy metals contents and other elements in the seaweed sample. The test parameters were Cadmium, Arsenic, Chromium, Iron, Manganese, Lead, Mercury, Nickel, Selenium and Zinc. All the test parameters were expressed in ppm.

Extraction

The ground seaweed sample (10 g) was extracted with 100 mL of methanol at room temperature for 72 h. The sample was then filtered with Whatman filter paper no. 1 and the solvent removed under vacuum at 40ºC using a rotary evaporator. The extract was then lyophilized overnight using freeze-dryer. Powdered form of extract is stored in -20ºC freezer for further use.

Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay (Kähkönen *et al*., 1999). Extracts (300 µL) were added into test tubes followed by 1.5 mL of Folin-Ciocalteu's reagent (10 times dilution) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorbance at 765 nm was measured. TPC was expressed as gallic acid equivalent (GAE) in mg per g of material.

DPPH

Radical scavenging activity was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (Miliauskas *et al*., 2004). Different dilutions of extract (1mL) were added to 2 mL of DPPH (5.9 mg/100mL methanol). The mixture was shake vigorously and stand in dark at room temperature for 30 minutes. Absorbance was measured at 517nm after 30 min. Radical-scavenging was calculated as IC_{50}.

Anti-tyrosinase activity

Anti-tyrosinase activity was determined using the modified Yen and Kim (2012) method. Firstly, 1.9 mL of 50 mM sodium phosphate buffer was mixed with 100 µL of sample extract solution and 20 µL of tyrosinase (1000 unit/mL in 50 mM phosphate buffer, pH 6.8) into a test tube. Next, 20 µL of 0.1 mM L-DOPA substrate was added in the test tube (Kamkaen *et al*., 2007). Then, the reaction mixture was incubated at 37ºC for 10 minutes in a water bath (Yoon *et al*., 2011). Absorbance was measured at 475 nm. The 50 mM sodium phosphate solution was used as a blank solution in spectrophotometer.

Results

In total of 8 elements (cadmium, arsenic, chromium, iron, manganese, lead, mercury, nickel and zinc) were tested on *E. cottonii* crude extract. Arsenic (3.9±0.002ppm), iron (14.9±0.001ppm) and zinc (3.0±0.0001ppm) were detected whereas cadmium, chromium, manganese, lead, mercury and nickel were not detected. By using Folin-Ciocalteu reagent method, TP content of *E. cottonii* crude extract was 3.40±0.013mg GAE/g extract. For DPPH scavenging activity assay, in total 5 different concentrations (10mg/mL, 20mg/mL, 30mg/mL, 40mg/mL and 50mg/mL) of *E. cottonii* crude extract were tested. A graph was plotted (Figure 1) and IC_{50} value of *E. cottonii* crude extract on DPPH was 38.82±0.99mg/mL. Tyrosinase enzyme inhibitory activity of *E. cottonii* crude extract was determined and the IC_{50} tyrosinase inhibition for *E. cottonii* crude extract was 234.33±21.85µg/mL.

Discussion and Conclusion

The eight tested elements there are only three elements were detected in *E. cottonii*, the detected elements are Arsenic (3.9 ppm), Iron (14.9 ppm) and Zinc (3.0 ppm). In general, arsenic is commonly found in seaweed due to the living habitat. This is the reason why marine organism has higher As contents than terrestrial organisms (Philip, 1990). Macroalgae such as seaweed tend to accumulate moderate amount of As and the concentrations are differ from brown, green and red seaweed. The result revealed that total As for *E. cottonii* is 3.9 ppm, although As was detected in *E. cottonii* but As appears in seaweeds is organic

arsenic which is non-toxic. Some of the seafood is found with very high concentrations of total As but it has no toxicity because most of the As is in Its organic forms (Tsuda *et al*., 1992). Besides, the result shows the iron content of E. cottonii is 14.9ppm. Iron is an important trace element for macroalgae growth. Supply of iron in coastal area is relatively high due to sources from sediment resuspension, urban inputs, and runoff. Thus, iron is rich in shallow coastal areas where usually macroalgae found. Thus this is why high amount of iron could be found in *E. cottonii.* The zinc content in *E. cottonii* is 3.0ppm. The uptake of Zn is influenced by the surrounding water or environment (Victoria *et al*., 2009).

Overdose of this element might harm to consumers. According to Food Safety Authority of Ireland (2009), arsenic appears in seaweeds is organic arsenic which is arsenosugars (dimethylarsinyl ribose derivatives), where it is considered to be virtually nontoxic. Meanwhile the daily consumption for inorganic arsenic is 0.002 mg/kg body weight, equivalent to 1.2 mg/day for a 60 kg adult. According WHO, daily consumption for Zinc and Iron are 8-11 mg/day and 8-18 mg/day. In general, the heavy metal contents were found to be low and the contribution of these heavy metals from *E. cottonii* when compared to the permissible tolerable weekly intake is negligible as suggested by WHO.

The total phenolic content (TPC) assay is used Folin-Ciocalteu (F-C) reagent to determine the presence of phenolic content within the sample. The TPC of the extract was referred to standard reference of Gallic acid (GA). The TPC value for the seaweed extract is 3.40±0.013mg GAE/g. E. cottonii is red seaweed which it can be found at intertidal region which is less sunlight. Compared to other species of seaweed such as brown and green seaweed which grow in areas where light is abundant. Increased of sunlight exposure has been correlated with increased of phenol content (José *et al*., 2013). Thus, this might be a reason that the TPC value for *E. cottonii* is lower compared to other species of seaweed such as brown and green seaweed where *E.cottonii* could be found at intertidal region which is less sunlight. According to Vijayabaskar and Shiyamala (2011), the phenolic content of brown seaweed *Turbinaria ornate* and *Sargassum wightii* are 47.32±1.63mg GAE/g extract and 35.98±1.75mg GAE/g extract respectively. According to Patricia *et al*. (2008), phenolic content of eight species of seaweed from north Borneo were compared. The eight species of seaweed consisting of three red seaweeds (*E. cottonii, E. spinosum* and *Halymenia durvillaei*), two green seaweeds (*Caulerpa lentillifera* and *C. racemosa*) and three

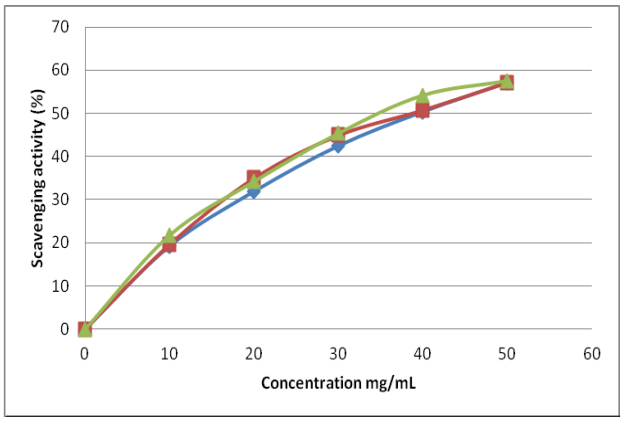

Figure 1. Scavenging activity of *E. cottonii* on DPPH

brown seaweeds (*Dicyota dichotoma, Sargassum polycystum* and *Padina* sp.) and they found TPC value for green seaweed and brown seaweed are higher than red seaweed. As compared to this two data, it shows clearly that total phenolic in red seaweed is lower than other seaweed such as brown seaweed and green seaweed.

The radical scavenging effect of DPPH was expressed in IC_{50} (Figure 1). The IC_{50} value for *E. cottonii* extract is 38.82±0.99 mg/mL. The scavenging activity of the sample on DPPH is strongly correlated with concentration. The scavenging effects on the DPPH radical increased when the concentration increasing, Lower IC_{50} value indicated higher antioxidative activity. According to Amin and Tan (2002), the free radical scavenging activity on DPPH was decrease in order of water then ethanolic extracts. In this study, the IC_{50} value showing a higher value which is 38.82±0.99 mg/mL compared to finding in Amin and Tan (2002). In year 2011, Walailuck *et al.* reported the DPPH radical of *Amphiroa* sp. *Halimeda macroloba, Sargassum binderi* and *Turbinaria conoides* are 7.827±0.120mg/mL, 0.837±0.002mg/mL, 20.147±0.000mg/mL and 0.128±0.002mg/mL respectively. As compared to *E. cottonii*, the IC_{50} value for this 4 species of seaweed is lower than *E. cottonii* which it also indicate that the antioxidant activity of *E. cottonii* is lower than the other 4 species of seaweed which reported by Walailuck *et al*. (2011). The result of DPPH radical is tally with result of total phenolic content which both antioxidant analysis shows a low antioxidant activity of *E. cottonii*. Different of growing environment and sunlight exposure might are positive correlated with antioxidant activity (Tomáš and Marie, 2010; José *et al*., 2013). Based on this study, *E. cottonii* was found low in antioxidant activity compared to other species of seaweed such as brown and green seaweed.

The anti-tyrosinase assay was conducted on *E. cottonii* extracts using L-DOPA as substrate and mushroom tyrosinase enzyme as contribute for

conversion of L-DOPA to dopachrome molecules. The percentage of tyrosinase inhibition activity for *E. cottonii* is 86% at 1000µg/mL. However, result elaborated in the form of 50% inhibition concentration (IC_{50}) is more convincing where the concentration of extracts required to achieve 50% tyrosinase inhibition could reflect the effective concentration of crude extract used. The mean of IC_{50} with standard deviations for methanol extract was 234.33±21.850µg/mL.

Moreover, there were various species of marine algae or seaweed worldwide that worth to identify its nutritional value and beneficial aspect to human health. For research purposes, the experiment starting material of seaweed is in dried form even fresh sample harvested will go through drying process to remove as much water molecules as possible. It is because the dry form sample enables to manipulate concentration used easily and water molecule would contain unknown substances interfere result (Jiménez *et al*., 2010 and Yen and Kim, 2012). According to Yen and Kim (2012) study, a type of red marine algae known as *Grateloupia lancifolia* was tested. The IC_{50} of tyrosinase inhibitory activities for *G. lancifolia* is 256 µg/mL. As compared to *E. cottonii, E. cottonii* shows a better anti-tyrosinase activity than *G. lancifolia*. The species, distribution area, living environment and harvesting condition could contribute to different strength of anti-tyrosinase properties between these two kinds of seaweeds.

Furthermore, there is plenty of anti-tyrosinase studied using various natural sources like fruits, edible flowers and etc. As compared to the other fruit and plant, *E. cottonii* show a better anti-tyrosinase activity than *Euphoria longana lam*. (3.2mg/mL) and *Chrysanthemum indicum* flower (9932.55µg/mL) (Rangkadilok *et al*., 2006; Rop et al., 2012).

As conclusion, the three detected elements are Arsenic (3.9ppm), Iron (14.9ppm) and Zinc (3.0ppm). Although Ar and Zn were detected in *E. cottonii* but it is non-toxic and safe to be consumed and it could be a good source of iron supply. Besides, low antioxidant activity is observed in *E. cottonii* due to relatively low TPC value (3.40±0.013mg GAE/g) and DPPH value (38.82±0.99mg/mL). Further research is required to verify the actual anti-tyrosinase compound within the seaweed. *E. cottonii* was shown a good anti-tyrosinase activity and it could be a good source of anti-tyrosinase activity which could be used in cosmetic product for whitening effect.

References

Amin, I. and Tan, S.H. 2002. Antioxidant activity of selected commercial seaweeds. Malaysian Journal of Nutrition 8(2): 167-177.

Chang, T.S. 2009. An updated review of tyrosinase inhibitors. International Journal of Molecular Sciences 10: 2440-2475.

Food Safety Authority of Ireland. 2009. Mercury, lead, cadmium, tin and arsenic in food. Toxicology Factsheet Series. Issue no.1, p1-13.

Jiménez, J.K., Connell, S.O., Lynos, H., Bradley, B. and Hall, M. 2010. Antioxidant, antimicrobial, and tyrosinase inhibition activities of acetone extract of *Ascophyllum nodosum*. Chemicals Papers 64(4): 434-442.

José, P., Nieves, L.D.M., Angela, P.M., Feliciano, P.C., Christina, D.C. and Brian, M.D. 2013. Sunlight exposure increases the phenolic content in postharvested white grapes. An evaluation of their antioxidant activity in *Saccharomyces cerevisiae*. Journal of Functional Foods 5(4): 1566-1575.

Kamkaen, N., Mulsri, N. and Treesak, C. 2007. Screening of some tropical vegetable for antityrosinase activity. Journal of Thai Pharmaceutical and Health Science 2(1): 15-19.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47: 3954-3962.

Kim, Y.J. and Uyama, H. 2005. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cellular and Molecular Life Sciences 62: 1707-1723.

Matanjun, P., Mohamed, S., Mustapha, N.M. and Muhammad, K. 2009. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii, Caulerpa lentillifera* and *Sargassum polycystum*. Journal of Applied Phycology 21: 75-80.

Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A. 2004. Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. Food Chemistry 85: 231-237.

Narayanaswamy, N., Rohini, S., Duraisamy, A. and Balakrishnan, K.P. 2011. Antityrosinase and antioxidant activities of various parts of *Mimusops elengi*: A comparative study. International Journal of Research in Cosmetic Science 1(1): 17-22.

O'Sullivan, L., Murphy, B., McLoughin, P., Duggan, P., Lawlor, P.G., Hughes, H. and Gardiner, G.E. 2010. Prebiotics from marine macroalgae for human and animal health applications. Marine Drugs 8(7): 2038-2064.

Özer, Ö., Mutlu, B. and Kivçak, B. 2007. Antityrosinase activity of some plant extracts and formulations containing ellagic acid. Journal of Pharmaceutical Biology 45(6): 519-524.

Patricia, M., Suhalia, M., Noordin, M.M., Kharidah, M. and Cheng. H.M. 2008. Antioxidant activities and

phenolics content of seaweeds from north Borneo. Journal of Applied Phycology 20: 357-373.

Petit, L. and Pierard, G.E. 2003. Skin-lightening products revisited. International Journal of Cosmetic Science 25: 169–181.

Phillips, D.J.H. 1990. Arsenic in aquatic organisms: a review, emphasizing chemical speciation. Aquatic Toxicology 16: 151-186.

Rangkadilok, N., Sitthimonchai, S., Worasuttayangkurn, L., Mahidol, C., Ruchirawat, M. and Satayavivad, J. 2006. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Journal of Food and Chemical Toxicology 45(2): 328-336.

Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J. and Vabkova, J. 2012. Edible flowers- A new promising source of mineral elements in human nutrition. Journal of Molecules 17: 6672-6683.

Tomáš, S., and Marie, K., 2010. Seasonal variation in total phenolic and flavonoid contents and DPPH scavenging activity of Bellis perennis L. flowers. Molecules 15: 9450-9461.

Tsuda, T., Babazono, A., Ogawa, T., Hamada, H., Mino, Y., Aoyama, H., Kurumanati, N., Nagira, T., Hotta, N., Harada, M. and Inomata, S. 1992. Inorganic arsenic: a dangerous enigma for mankind. Applied Organometallic Chemistry 6: 309-322.

Velioglu, Y.S., G. Mazza, L. Gao. and B.D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 46: 4113−4117.

Victoria, B., José, M.A., Fernando, S. and Juan, J.G. 2009. Heavy metals in edible seaweeds commercialized for human consumption. Journal of Marine Systems 75(1-2): 305-313.

Vijayabaskar, P. and Shiyamala, V. 2011. Antibacterial activities of brown marine algae (*Turbinaria ornate* and *Sargassum wightii*) from the gulf of Mannar biosphere reserve. Journal of Biological Research 5(2): 99-102.

Walailuck, B., Yuwadee, P., Duangta, K., Jeeraporn, P., Doungporn, A., Chayakorn, P., Prasan, S. and Panmuk, V. 2011. Antimicrobial and anti-inflammatory properties of various seaweeds from the Guf of Thailand. Journal of Agricultural and Biology 13: 100-104.

Yen, H.N. and Kim, S.M. 2012. Antioxidative, anticholinesterase and antityrosinase activities of the red alga *Grateloupia lancifolia* extracts. African Journal of Biotechnology 11(39): 9457-9467.

Yoon, K.N., Alam, N., Lee, J.S., Lee, K.R. and Lee, T.S. 2011. Detection of Phenolic compounds concentration and evaluation of antioxidant and antityrosinase activities of various extracts from the fruiting Bodies of *Lentinus edodes*. Journal of World Applied Science 12(10): 1851-1859.