ปริมาณฟีนอลิกรวมและฤทธิ์ขจัดอนุมูล DPPH ของสาหร่ายทะเล 6 ชนิด จากชายฝั่งภาคใต้ของประเทศไทย

Total phenolic contents, DPPH radical-scavenging activities of six seaweeds from the southern coast of Thailand

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บทคัดย่อ

นำสาหร่ายทะเล 6 ชนิดจากชายฝั่งภาคใต้ของประเทศไทย ได้แก่ สาหร่ายใบ(*Pyropia vietnamensis*) สาหร่ายผมนาง (*Gracilaria tenuistipitata*) สาหร่ายพวงองุ่น (*Caulerpa macrophysa*) สาหร่ายเม็ดพริก (*Caulerpa microphysa*) สาหร่ายผักกาดทะเล (*Ulva lactuca*) และสาหร่ายทุ่น [*Sargassum* sp. (SG-0044)] มาสกัดด้วยเมทานอลที่เข้มข้นต่างๆ กัน คือ 0%, 25%, 50% และ 100% นำสารสกัดที่ได้ไปหาปริมาณสารประกอบฟีนอลิกรวม (TPC) และความสามารถในการขจัดอนุมูลอิสระโดยใช้ DPPH พบว่า การสกัดสาหร่ายใบ และสาหร่ายทุ่น ด้วยน้ำ (เมทานอล 0%) ให้ปริมาณ TPC และค่าความสามารถขจัดอนุมูล DPPH สูงกว่าการสกัดด้วยเมทานอล 25%, 50% และ 100% ตามลำดับ ในขณะที่ สารสกัดสาหร่ายพวงองุ่นมีปริมาณ TPC และความสามารถขจัดอนุมูล DPPH เพิ่มขึ้นตามความเข้มข้นของเมทานอล โดยสารสกัดด้วยน้ำของสาหร่ายใบซึ่งมี TPC ในปริมาณสูงแสดงฤทธิ์ต้านอนุมูลอิสระได้ดีที่สุดเมื่อเทียบกับสารสกัดทั้งหมด ผลวิเคราะห์ค่าสัมประสิทธิ์ความสัมพันธ์ระหว่างปริมาณ TPC กับความสามารถขจัดอนุมูลอิสระพบว่า สารสกัดสาหร่ายใบ (R^2 = 0.9483) สาหร่ายพวงองุ่น (R^2 = 0.9028) และสาหร่ายทุ่น (R^2 = 0.8422) มีความสัมพันธ์กันในเชิงบวก ในขณะที่สารสกัดสาหร่ายชนิดอื่นไม่มีความสัมพันธ์ดังกล่าว ข้อมูลทั้งหมดแสดงให้เห็นว่าสารสกัดสาหร่ายใบด้วยน้ำมีประสิทธิภาพในการขจัดอนุมูลอิสระสูงสุด ซึ่งเป็นผลจากการออกฤทธิ์ของ TPC จำพวกมีขั้วสูง (polar phenolics) เป็นส่วนใหญ่ รองลงมาคือสารสกัดเมทานอลของสาหร่ายพวงองุ่นซึ่งน่าจะเป็นเพราะสารออกฤทธิ์จำพวกมีขั้วต่ำหรือไม่มีขั้ว (non-polar phenolics) ในขณะที่ TPC ของสารสกัดสาหร่ายที่เหลืออีก 4 ชนิด แทบไม่มีฤทธิ์ต้านออกซิเดชันดังกล่าว ดังนั้น TPC ในสารสกัดจากสาหร่ายใบ สาหร่ายทุ่น และสาหร่ายพวงองุ่น มีประสิทธิภาพสูงในการขจัดอนุมูลอิสระ แต่ TPC ในสารสกัดจากสาหร่ายผมนาง สาหร่ายเม็ดพริกและสาหร่ายผักกาดทะเล ไม่มีประสิทธิภาพในการขจัดอนุมูลอิสระ

คำสำคัญ : สาหร่ายทะเล สารต้านอนุมูลอิสระ

Abstract

Six species of seaweed from the southern coast of Thailand, including *Pyropia vietnamensis*, *Gracilaria tenuistipitata, Caulerpa macrophysa, Caulerpa microphysa, Ulva lactuca* and *Sargassum* sp. (SG-0044) were extracted with methanol at different concentrations (0%, 25%, 50% and 100%). The extracts were examined for their total phenolic contents (TPC) and antioxidant activities by DPPH radical scavenging assay. The aqueous (0% methanol) extracts of *P. vietnamensis*, *U. lactuca* and *Sargassum* sp. showed higher phenolic contents and antioxidant activities than their corresponding methanol extracts (25, 50 and 100 %), in contrast to those of *C. macrophysa*. When we compared the results of the phenolic compounds and the antioxidant activities, the high correlation coefficients were found in *P. vietnamensis* (R^2 = 0.9483), *C. macrophysa* (R^2 = 0.9028) and *Sargassum* sp. (R^2 = 0.8422) whereas the rest of them showed no correlations. Therefore, TPC were the most effective antioxidants in *P. vietnamensis*, *Sargassum* sp. and *C. macrophysa* but not effective antioxidants in *G. tenuistipitata, C. microphysa* and *U. lactuca*.

Keywords : Phenolic DPPH Seaweeds

Introduction

Reactive oxygen species or free radicals are by-products generated during oxidative metabolism in living organisms. They are responsible for aging and causing various human diseases (Parke, 1999). Many studies show that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. Antioxidants have become a topic of increasing interest. Several marine algal extracts have been demonstrated to possess strong antioxidant properties (Zubia *et al*., 2007; Yangthong *et al*., 2009; Boonchum *et al*., 2011). The potential antioxidant compounds derivable from seaweed were identified under two groups, pigments (e.g. fucoxanthin, astaxanthin, carotenoid) and polyphenols (e.g. phenolic acid, bromophenols, pholotanins, flavonoids, tannins). A high correlation between the total phenolic content and antioxidant activity has been reported by many researchers (Siriwardhana *et al*., 2003; Chew *et al*., 2008; Wang *et al*., 2008). Phenolic compounds can act as chain-breaking antioxidants by donating hydrogen to free radicals and thereby producing relatively un-reactive antioxidant radicals. The phenol rings in phenolic compounds act as electron traps and are responsible for the multifunctional antioxidant properties such as the scavenging of hydroxyl radicals, peroxy radicals or superoxides. The metal chelating potency of phenolic compounds has been reported to be dependent upon their unique phenolic structure and the number and location of their hydroxyl groups (Santoso *et al*., 2004). Polyphenols

from terrestrial plants are derived from gallic and ellagic acid, whereas algal polyphenols are derived from polymerized phloroglucinol units. Phlorotannins are a group of phenolic compounds. Algal phlorotannins have up to eight interconnected rings and are thus more potent antioxidants than plant polyphenols (Wang *et al*., 2008). Seaweed polyphenols such as phlorotannins have been reported to scavenge free radicals, superoxide radicals (Kuda *et al*., 2005), peroxyl radical (Wang *et al*., 2008), and chelate ferrous ions (Chew *et al*., 2008). The antioxidant activity of the extracts produced is significantly affected by the nature of the seaweed matrix and extracting solvent. Polar solvent as pure methanol or as aqueous mixtures are recommended for the extraction of phenolic antioxidant components from a plant material and seaweed (Anwar *et al*., 2010, Boonchum *et al*., 2011, Chew *et al*., 2008). The methanol-water mixture has high polarity and thus greater efficacy towards the extraction of polar phytochemicals such as phenolics (Anwar *et al*., 2010).

Thailand is located in a tropical area of South-East Asia with a wide variety of marine algae. In the southern part of the country, *Pyropia vietnamensis*, *Gracilaria tenuistipitata, Caulerpa macrophysa, Caulerpa microphysa, Ulva lactuca* and *Sargassum* sp. are distributed along the coasts of the Gulf of Thailand and Andaman Sea. They are commonly used for human consumption. *P. vietnamensis* is a red alga that is known under a local name “Sarai Bai”. It was found on the rocks of upper tidal zone along the coasts of Songkhla during monsoon season in December to February (Tsutsui *et a*l., 2012). *G. tenuistipitata* is also a red algae, its thalli forming hair like filaments. This seaweed contains high quality agar (Lewmanomont and Chirapart, 2004) and biologically active sulphated, galactose-based polysaccharides with antibacterial and antiviral properties (Bansemir *et al*., 2006). *C. macrophysa*, *C. microphysa* and *U. lactuca* are green algae of the division Chlorophyta. *C. macrophysa* is called sea grape. It is similar to *C. microphysa,* having the branchlets without constrictions between the base of the spherical head and the stalks. The branchlets of *C. macrophysa*, however, are bigger than those of *C. microphysa*. This seaweed forms clumps on rocks in mid-intertidal to subtidal zones along shorelines with calm to moderate water movement and in tidal pools (Tsutsui *et al*., 2012). *U. lactuca* is also known by the common name sea lettuce. It is a potentially rich source of natural colorants which can be utilized in food and pharmaceutical industries (Abd El-Baky *et al*., 2008). *Sargassum* sp. is a brown seaweed. Numerous species are distributed throughout the temperate and tropical oceans of the world. It is commonly known as gulf-weed. This type of seaweed is used in producing medicines for humans, and fertilizers. It has biologically active fucose-based polysaccharides in abundant (Matsukawa, 1997; Yan *et al*., 1999; Zhang *et al*., 2005). In this study, we evaluated the efficacy of methanol at different concentrations in extracting DPPH radical scavengers from the six species of

seaweeds. The correlations between their antioxidant activities and total phenolic contents were also determined.

Materials and methods

Materials

Marine algae of six species were collected during October to December 2008 from the coastal areas of the southern part of Thailand. *P. vietnamensis* was collected from Kao Seng Beach in the Gulf of Thailand, Songkhla Province. *G. tenuistipitata* and *U. lactuca* were collected from Pattani Bay in the Gulf of Thailand, Pattani Province. *C. macrophysa* and *C. microphysa* were collected from Sah-rai Island in Andaman Sea, Satun Province. *Sargassum* sp. was collected from between Cat Island and Rat Island in the Gulf of Thailand, Songkhla Province. Once harvested, they were stored in plastic bags and placed on ice for transport to the laboratory.

Preparation of extracts

Fresh seaweeds were washed thoroughly with running tap-water to remove salts, sand and epiphytes. They were dried at 55 °C and then milled. The milled seaweeds were subsequently used for preparing the extracts. As modified from those of Chew *et al*. (2008), one gram of the milled seaweed of each species was extracted with 50 ml of different concentrations of methanol (0%, 25%, 50% and 100%) by continuously shaking for 1 h. The extract was obtained after filtration with filter paper. It was left at room temperature until completely dried and then stored at -20 °C for further analysis.

Measurement of total phenolic contents

The total phenolic content (TPC) of each extract was measured using Folin-Ciocalteu method as described by Velioglu *et al.* (1998). Briefly, 0.75 ml of diluted Folin-Ciocalteu reagent (1:9 v/v; Folin-Ciocalteu reagent: distilled water) was mixed with 100 µl of sample (5 mg ml^{-1}) and was left at room temperature for 5 minutes. Then 0.75 ml of 10% sodium carbonate solution was added, followed by 10 ml of distilled water. After mixing, it was allowed to stand at room temperature for 90 minutes. The TPC was determined using a spectrophotometer at 725 nm. Tannic acid was used as the standard, and the TPC was expressed in terms of mg tannic acid equivalents (TAE) per 100 g of dried samples.

Measurement of antioxidant activities

The DPPH (1,1-diphenyl-1-picrylhydrazyl) radical scavenging activity of the seaweed extract was measured by the method described by Hutadilok-Towatana *et al*. (2006). Briefly, 0.6 ml of 0.2 mM DPPH in methanol was added to samples of 0.3 ml of the different seaweed extracts in water. The mixtures was shaken vigorously then allowed to stand at room temperature for 30 minutes before the

absorbance was read at 518 nm. Butylated hydroxytoluene (BHT) was used as a positive control. The capability to scavenge the DPPH radical was calculated using the following equation: Scavenging (%) = $[(1 - A/A_0) \times 100]$, where A_0 is the absorbance of DPPH alone, and A is the absorbance of the reaction mixture containing DPPH and sample.

Statistical analysis

Data were expressed as the means $\pm$ SD of three measurements and analyzed using one-way ANOVA by Duncan test. Differences in mean values were considered significant when $P<0.05$. The correlation between TPC and DPPH radical were investigated with correlation coefficients (R^2) analysis.

Results

Total phenolic content

As shown in Table 1, the aqueous (0% methanol) extracts of *P. vietnamensis*, *U. lactuca* and *Sargassum* sp. had higher TPC than those extracted with methanol. In contrast, the TPC of *C. macrophysa* and *C. microphysa* extracts with 100% methanol were significantly higher than those treated with methanol at lower concentrations. Among them, the 100% methanol extract of *C. macrophysa* showed the highest TPC at 841.18 $\pm$ 14.34 mg TAE 100 g^{-1} dried sample. The TPC values of *G. tenuistipitata* extracts, however, were not different from each other.

DPPH radical scavenging activity

The aqueous extracts of *P. vietnamensis* and *Sargassum* sp exhibited higher DPPH radical scavenging abilities than their methanol ones. These results, however, were in reverse to those of *C. macrophysa*. Among our six algae, *P. vietnamensis* exhibited the most potent antioxidant activity. Its extracts showed the methanol concentration-dependent effects with 78-68% DPPH inhibition found in 0-50% methanol treatments. In comparison, the brown seaweed *Sargassum* sp. extracts also revealed the methanol concentration-dependent but rather poor anti-DPPH effects, whereas the others were considered to be ineffective.

Table 1 Total phenolic content of methanol extracts from various seaweeds

	TPC (mg TAE 100 g^{-1} dried samples)[1]			
	0 % methanol	25 % methanol	50 % methanol	100 % methanol
P. vietnamensis	549.64 ± 40.91^{c}	534.32 ± 26.41^{c}	416.56 ± 30.87^{b}	104.44 ± 11.58^{a}
G. tenuistipitata	191.53 ± 67.10	159.82 ± 31.87	123.11 ± 33.06	109.34 ± 21.89
C. macrophysa	133.15 ± 73.25^{a}	144.15 ± 20.56^{a}	174.30 ± 38.27^{a}	841.18 ± 14.34^{b}
C. microphysa	187.12 ± 27.90^{b}	145.31 ± 18.96^{a}	150.71 ± 24.94^{a}	235.13 ± 4.69^{c}
U. lactuca	157.17 ± 21.46^{c}	156.39 ± 13.12^{c}	117.29 ± 20.94^{b}	61.21 ± 21.30^{a}
Sargassum sp.	152.52 ± 0.57^{c}	80.31 ± 4.51^{b}	42.13 ± 3.62^{a}	42.69 ± 4.18^{a}

[1]Mean ± standard deviation of three replications

Means within each row not sharing a common superscript are significantly different ($p<0.05$)

Table 2 DPPH radical scavenging activity of methanol extracts from various seaweeds

	(%) DPPH radical scavenging activity[1]			
	0 % methanol	25 % methanol	50 % methanol	100 % methanol
P. vietnamensis	78.29 ± 0.72^{d}	76.07 ± 1.12^{c}	67.97 ± 2.02^{b}	20.13 ± 1.13^{a}
G. tenuistipitata	na	na	3.14 ± 0.59	11.71 ± 2.94
C. macrophysa	9.91 ± 0.34^{a}	10.58 ± 0.61^{a}	13.84 ± 0.76^{b}	22.52 ± 1.90^{c}
C. microphysa	1.63 ± 0.31^{b}	4.47 ± 0.65^{c}	0.34 ± 0.33^{a}	1.44 ± 0.80^{b}
U. lactuca	na	na	na	5.11 ± 0.36
Sargassum sp.	16.58 ± 0.94^{b}	2.24 ± 0.92^{a}	2.16 ± 0.03^{a}	1.88 ± 0.91^{a}

[1]Mean ± standard deviation of three replications, na = no activity.

Means within each row not sharing a common superscript are significantly different ($p<0.05$)

Correlation between the DPPH radical scavenging activity and total phenolic contents

The correlation coefficients (R^2) between the DPPH radical scavenging activity and TPC of the seaweed extracts were determined (Figure 1). The DPPH radical scavenging activities and TPC of *G. tenuistipitata, C. microphysa* and *U. lactuca* were not correlated as revealed by R^2 (0.1582, 0.0642, and 0.3094, respectively) whereas those of *P. vietnamensis*, *C. macrophysa* and *Sargassum* sp were significantly correlated (R^2 of 0.9483, 0.9028, and 0.8422, respectively). Therefore, these results indicate that antioxidant capacities of *P. vietnamensis*, *C. macrophysa*, and *Sargassum* sp. are determined by their phenolic contents.

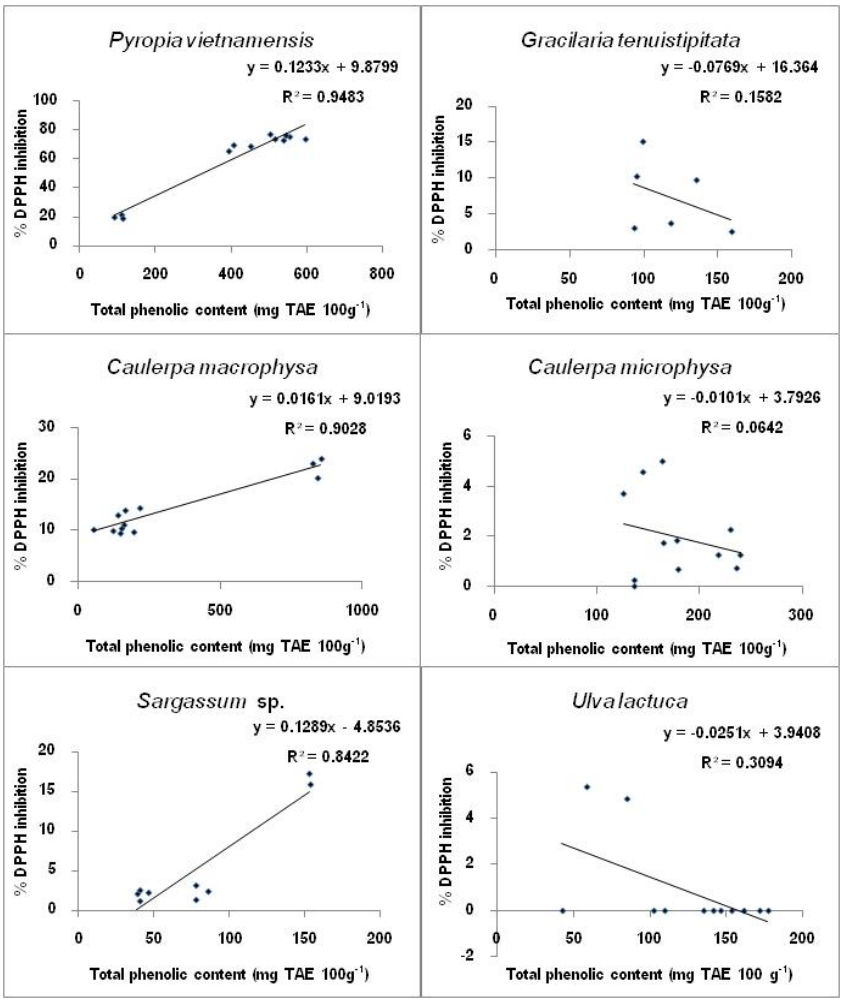

Figure 1 Correlations between the DPPH radical scavenging activity and total phenolic content (TPC) of each seaweed extract.

Discussion

Phenolic compounds play a major role in the protection of oxidation processes which can act as free radical scavengers, hydrogen donators, metal chelators and singlet oxygen quenchers (Puerta, 1999). The major active compounds in different seaweed extracts have been reported to be phlorotannin and bromophenol (Nakai *et al*., 2006; Kuda *et al*., 2007; Li *et al*., 2007). Phenolic compounds categorized into classes depending on their structure and subcategorized within each class according to the number and position of hydroxyl group and the presence of other substituents. Generally, green and red seaweeds have low concentrations of phenols compared to brown seaweed species (Mabeau and Fleurence, 1993; Matanjun *et al*., 2008). Polyphenols (fucol, fucophlorethol, fucodiphloroethol G, and ergosterol) and the phenolic compound phlorotannin are abundant in brown

macroalgae, such as *Sargassum mangrevense, Ascophyllum nodosum*, and *Fucus spiralis* (Cerantola *et al*., 2006; Zhang *et al*., 2007; Zubia *et al*., 2008). Contradictory results were arisen from this study. The TPC of brown seaweed (*Sargassum* sp.) was lower than those of green seaweeds (*C. macrophysa*, *C. microphysa* and *U. lactuca*) and red seaweeds (*P. vietnamensis*, *G. tenuistipitata*). The findings that TPC in our seaweeds except for *C. macrophysa* and *C. microphysa* were more extractable with aqueous solvent also suggest their high contents of polar compounds.

The main phenolic compound of marine origin is bromophenol. The levels of bromophenol in *U. lactuca* dramatically increased at the end of the summer whilst decreasing during the rest of the year (Connan *et al*., 2004). Heo and co-workers (2005) used enzymatic extracts from seaweeds as a potential natural water-soluble source of antioxidants to confirm free radical, superoxide anion, hydroxyl radical and hydrogen peroxide scavenging activities as well as an inhibitory effect on DNA damage. Some seaweed enzymatic extracts indicated relatively higher antioxidant activities, as compared to commercial antioxidants such as α-tocopherol, butylated hydroxyanisole (BHA), and BHT. Cho and co-workers (2007) reported that ethanol extracts of *S. siliquastrum* exhibited a 95% scavenging effect of DPPH radicals at 0.5 mg ml^{-1} or greater. Bromophenol extracted from brown seaweed using water had the highest antioxidant activity compared to ethanol extracts (Farvin *et al*., 2010). Our findings that the aqueous extracts of brown (*Sargussum* sp.) and red (*P. vietnamensis*) algae could inhibit more DPPH radicals than their methanol ones thus suggest the anti-oxidative role of polar phenolic compounds including bromophenol in both seaweeds.

DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. DPPH assay has been used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors (Singh and Rajini. 2004). Scavenging of DPPH radical is the basis of the popular antioxidant assay (Sharma and Bhat, 2009). Several studies have indicated strong correlation between the TPC and antioxidant activity (Zubia *et al*., 2007; Yangthong *et al*., 2009; Boonchum *et al*., 2011), which is in agreement with *P. vietnamensis*, *Sargassum* sp. and *C. macrophysa* extracts in this study. Although TPC in *C. macrophysa* was found to be markedly increased when extracted with methanol alone (about 8 folds of the aqueous extract), anti-DPPH activity measured in the 100% methanol extract of this seaweed was not as high as its TPC level. This indicates that phenolic compounds, mostly non-polar, obtained from pure methanol extraction of *C. macrophysa* would not act as antioxidant.

The low antioxidant activities and no correlations between TPC and the activities in *G. tenuistipitata, C. microphysa* and *U. lactuca* both indicate that phenolic compounds may not play anti-

oxidative role in these seaweeds. Similar to our cases, Cho and co-workers (2011) found non-significant correlation between anti-DPPH activity and TPC of the extract of *U. prolifera*. They also suggested that the strong antioxidant activity of the *U. prolifera* would come from a chlorophyll compound, pheophobid rather than phenolic compounds. More recently, Farasat and co-workers (2013) reported correlations between antioxidant activities by DPPH and flavonoids in the extracts of *U. clathrata* and *U. prolifera* and thus suggested the contribution of flavonoids as main antioxidants in these *Ulva* species.

Conclusions

In this study, *P. vietnamensis* with high phenolic content showed the strongest antioxidant activity *in vitro*. A strong relationship between the antioxidant capacity (DPPH) and TPC of *P. vietnamensis*, *Sargassum* sp., and *C. macrophysa* extracts also demonstrated the anti-oxidative role of phenolic compounds in these marine seaweeds, thus indicating their possible benefits on human health when consumed. Among the tested materials, *P. vietnamensis* presented the most potent antioxidant activity. Further study is needed to identity active phytochemicals contained in this red seaweed.

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