ฤทธิ์กำจัดอนุมูลอิสระและการจับตัวกับเหล็กของสารสกัดจากฟองน้ำทะเลไทย

Free Radical Scavenging and Ferrous Chelating Activities of Thai Marine Sponge Extracts

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บทคัดย่อ

ศึกษาฤทธิ์การต้านอนุมูลอิสระของสารสกัดจากฟองน้ำทะเลไทย 23 ชนิด โดยเปรียบเทียบฤทธิ์การต้านอนุมูลอิสระด้วยตัวทำละลาย 3 ชนิด พบว่าสารสกัดด้วยเมธานอลออกฤทธิ์กำจัดอนุมูลอิสระ DPPH และการจับตัวกับเหล็กได้ดี ส่วนสารสกัดด้วยเมธานอลและอะซีโตนออกฤทธิ์กำจัดอนุมูลอิสระ ABTS ได้ดี และสารสกัดด้วยไดคลอโรมีเทนส่วนใหญ่ออกฤทธิ์ต้านอนุมูลอิสระน้อยที่สุด สารสกัดด้วยเมธานอลจากฟองน้ำ *Hyrtios* sp.1 มีฤทธิ์กำจัดอนุมูลอิสระ ABTS และ DPPH ดีที่สุดที่ IC_{50} เท่ากับ 77.14 และ 228.9 µg/ml และสารสกัดด้วยไดคลอโรมีเทนจากฟองน้ำ *Stylissa* sp. มีฤทธิ์จับตัวกับเหล็กดีที่สุดที่ IC_{50} เท่ากับ 159.4 µg/ml และยังพบว่าฟองน้ำ *Hyrtios* sp.1 มีปริมาณฟีนอลสูงที่สุดคือ 124.27± 3.73 mgGAE/กรัมสารสกัด ซึ่งคาดว่าฟีนอลในฟองน้ำชนิดนี้น่าจะทำหน้าที่ออกฤทธิ์กำจัดอนุมูลอิสระ

คำสำคัญ : ฟองน้ำ การกำจัดอนุมูลอิสระ การจับตัวกับเหล็ก

Abstract

Twenty-three samples of Thai marine sponges were evaluated for antioxidant activities by comparing the effect of three solvents on the antioxidant activities. Almost of methanolic extracts displayed high DPPH radical scavenging and ferrous chelating activity while methanolic and acetone extracts displayed high ABTS radical scavenging activity. Dichloromethane extracts almost displayed low antioxidant activities. The most significant ABTS and DPPH scavenging activity were determined for the methanolic extract from *Hyrtios* sp.1 at IC_{50} value of 77.14 and 228.9 µg/ml, respectively. The dichloromethane extract from *Stylissa* sp. had the most significant ferrous chelating activity at IC_{50} value of 159.4 µg/ml. *Hyrtios* sp.1 had the highest phenol content at the value of 124.27±3.73 mgGAE/g of extract which supposed to perform its radical scavenging activities.

Keywords : sponge, free radical scavenging, DPPH, ABTS, ferrous chelating

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Introduction

Nowadays, the daily life of human are encountered to various toxic food, UV radiation and pollutants which caused serious diseases almost due to the oxidation stress in the body. The oxidation processes produce various type of free radicals such as superoxide anion, singlet oxygen and hydroxyl radicals along with peroxides and transition metal. Oxidation induced by free radicals or metals have degenerate effect to living cells and cause serious health problems such as cancer, atherosclerosis, cardiovascular diseases, hypertension, diabetes, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), rheumatoid arthritis, and ageing. Free radicals are a primary cause or a downstream consequence of tissue injury. The physiological functions in human body will inhibit the oxidation products by using antioxidants agents. The antioxidants are free radical scavengers or metal chelating which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (Papas, 1999; Valko *et al*., 2007). The antioxidants from natural sources play an important role in preventing oxidation products and are in need due to the emerging of toxic synthetic antioxidants (Takamatsu *et al*., 2003).

Marine sponges are reported as a rich source of active antioxidants comparing to the commercial chemical antioxidants such as ascorbic acid, vitamin E, butylated hydroxyanisole (Kim & Dewapriya, 2012). There are some literatures involving natural compounds from marine sponges with various antioxidative mechanisms. The mechanisms of antioxidative activities from marine sponges have been reported, for example scavenging activity of free radicals; DPPH, ABTS, nitric oxide (NO) and superoxide (SO) (Utkina *et al.,* 2004; Utkina, 2013; Aktas *et al*., 2013; Youssef *et al.,* 2013) total antioxidant activity (Rivera & Uy, 2012) inhibition of linoleic acid (Utkina*,* 2009) oxygen radical absorbance capacity (ORAC) (Nguyen *et al.,* 2012) and metal chelating activity (Taheri & Jalalinezhad, 2015). The active metabolites from sponges that act as antioxidants such as indole, aromatic alkaloid, polyketide, terpene and phenol have been well documented (Utkina *et al.,* 2004; Chairman *et al*., 2012; Kim & Dewapriya, 2012; Nguyen *et al.,* 2012; Aktas *et al*., 2013).

The natural products from sponges are promising sources of various bioactive compounds for clinical application (Selvin & Lipton, 2004; Roopesh, 2007; Mehbub *et al.,* 2014). There are some literatures reported the potency of marine sponges collected from Thailand coast with various activities such as antibacterial, antimalarial (Pedpradab *et al*., 2010), antifungal (Wattanadilok *et al*., 2007), cytotoxic (Phuwapraisirisan *et al*., 2003; Mahidol *et al*., 2009; Prawat *et al*., 2011), anticancer (Kijjoa *et al*., 2007) and anticholinesterase (Nukoolkarn *et al*., 2008). Although many bioactivities have been discovered in sponges collected from Thailand coast, the study referred to antioxidants are limited.

The coasts of Thailand both Gulf of Thailand and Andaman sea are furnished with diverse marine sponges (Hongpadharakiree *et al*., 2011; Hongpadharakiree & Putchakarn, 2013). Some Thai marine sponges were known to produce a wide variety of bioactive compounds, anyway the study for antioxidants activity are relatively few. In this study we assessed antioxidant activity of marine sponges collected from

Thailand coast via DPPH, ABTS radical scavenging activity and ferrous chelating activity. The effects of different solvents for extraction were compared for antioxidant activity. The phenol and flavonoid content which supposed to act the antioxidant were also evaluated.

Methods

Sample collection : Twenty three marine sponges were collected from Gulf of Thailand (east coast), Ko Lan, Cholburi ($12^{o}53^{/}56.65^{//}$N,$100^{o}46^{/}37.34^{//}$E) and from Andaman sea (west coast) , Mu Koh Kam, Ranong province ($9^{o}27^{/}18.5^{//}$N, $098^{o}19^{/}48.9^{//}$E) by SCUBA diving during February-April, 2015 (Table 1). The voucher specimens were preserved in 70% ethanol for identification by the specialist at Museum of Fisheries (Natural History), Faculty of Fisheries, Kasetsart University.

Sample preparation : The samples were removed the fouling organisms and sediments, then immersed into freshwater and air dried for 1 hr. After that the samples were further lyophilized (SCANVAC) and grounded to a fine powder kept in the -20^{o}C freezer.

Sample extraction : Initially, all the sponges were screened for antioxidant activity by using the supernatants. The supernatants were prepared by weighing 3 mg of lyophilized samples and extracted with 3 ml of methanol, sonicated (SONICS : VCX750) for 1 min and centrifuged (NUVE : NF1200) at 3,000 rpm for 10 min. The supernatants at concentration of 1mg/ml were pipeted out and kept in vials for further antioxidant evaluation. The samples which showed the radical scavenging activity and ferrous chelating ability higher than 90 % were subsequently screened. After that the crude extracts were prepared by using two grams of samples extracted with 20 ml of three different solvents; methanol, acetone and dichloromethane. The extracts were sonicated for 3 min, centrifuged at 3,000 rpm for 10 min then concentrated under reduced pressure using rotary evaporator (Buchi : R200), finally yielded crude extracts. The crude extracts were stored at 4^{o}C in air-tight vials for antioxidant assays. The crude extracts were dissolved in DMSO and evaluated for the effect of different extraction solvents on the antioxidant activity and for the study of antioxidant efficacy by IC_{50} values which in the concentration between 0.49-1,000 µg/ml.

DPPH radical scavenging assay : In this method a stable free radical, DPPH (2, 2 –diphenyl-1 picrylhydrazyl) was reduced by the antioxidant agents, with donation the electron, resulting decolorization from blue to yellow color. This assay was applied according to Yamazaki *et al*. (1994) and Chatatikun and Chiabchalard (2013). The supernatants and crude extracts along with ascorbic acid which used as standard control were pipeted 20 µl into 96 well-plate with 3 replications. Then 180 µl of DPPH (0.016mM in methanol) were added. The mixtures were maintained in the darkness for 30 min and the absorbances were measured by microplate reader (Biochrome : EZ Read 400) at 540 nm. The DPPH radical scavenging activity of the extracts were calculated by equation (1)

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_{sample} - A_{sample\ blank})/A_{control}] \times 100 \quad (1)$$

A_{sample} = The absorbance of extracts with DPPH

$A_{sample\ blank}$ = The absorbance of extracts only

$A_{control}$ = The absorbance of DPPH without extracts

ABTS radical scavenging assay : This assay was performed by using 2,2/-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) which was generated by oxidation of ABTS with potassium persulfate and was reduced in the presence of such hydrogen-donating antioxidants. This assay was applied according to Re *et al.* (1999) and Chatatikun and Chiabchalard (2013). The supernatants and crude extracts along with ascorbic acid which used as standard control were pipeted 20 µl into 96 well-plate with 3 replications. Then 180 µl of ABTS (0.7mM ABTS mixed with 2.45 mM potassium persulfate and given the absorbance at 0.07±0.02 at 750 nm) were added. The mixtures were kept in the darkness for 30 min and the absorbances were measured using microplate reader at 750 nm. The ABTS radical scavenging activity of the extracts were calculated by equation (2)

$$\text{ABTS radical scavenging activity (\%)} = [1-(A_{sample} - A_{sample\ blank})/A_{control}] \times 100 \quad (2)$$

A_{sample} = The absorbance of extracts with ABTS

$A_{sample\ blank}$ = The absorbance of extracts only

$A_{control}$ = The absorbance of ABTS without extracts

Ferrous chelating assay : Ferrozine is a compound that can quantitavely form stable magenta-coloured complexes with ferrous ion (Fe^{2+}). In the presence of other chelating agents, the complex formation is disrupted and the colour of the complex decreases. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator. This assay was applied according to Kellog and Lila (2013). 135 µl of distilled water were added into 96 well plate then 5 µl of 2mM ferrous chloride, 10 µl of supernatants and crude extracts along with EDTA as standard control and finally 10 µl of 5mM ferrozine were added. The well plates were maintained for 10 min and the absorbances at 570 nm were measured using microplate reader. The ferrous chelating activity of the extracts were calculated by equation (3)

$$\text{Ferrous chelating activity (\%)} = [1-(A_{sample} - A_{sample\ blank})/A_{control}] \times 100 \quad (3)$$

A_{sample} = The absorbance of extracts with ferrozine

$A_{sample\ blank}$ = The absorbance of extracts only

$A_{control}$ = The absorbance of ferrozine without extracts

Total phenolic content : The phenolic contents were analyzed by Folin-Ciocalteu method according to Chatatikun and Chiabchalard (2013). The assay was done in 96 well plate by adding 50 µl of distilled water, 50 µl of crude extracts, 50 µl of 10% Folin-Ciocalteu reagent, finally 50 µl of 1M sodium carbonate. The mixtures were incubated in darkness for 60 min. The absorbances were measured at 750 nm by microplate reader. The amounts of phenol were calculated by comparing with the standard curve of gallic acid which prepared at the concentration of 5, 10, 25, 50 and 100 µg/ml and given the unit as mg equivalent to gallic acid (mg GAE/g extract)

Total flavonoid content : The method of flavonoid contents were according to Chang *et al*. (2002). The assay was done in 96 well plate by adding 10 µl of 10 % aluminium chloride, 50 µl of crude extracts, 50 µl of 95% ethanol finally 50 µl of 1M sodium acetate. The mixtures were incubated in darkness for 40 min. The absorbances were measured at 405 nm by microplate reader. The amounts of flavonoid were calculated by comparing with the standard curve of quercetin which prepared at the concentration of 5, 10, 25, 50 and 100 µg/ml and given the unit as mg equivalent to quercetin (mg QTE/g extract)

TLC identification : The active extracts were analyzed for the functional group which supposed to showed the radical scavenging activity and ferrous chelating activity by TLC. The extracts were diluted with DMSO and applied on 5x5 cm TLC plates (Si60,F254) eluted with chloroform and methanol at 5 : 1. The active spot could be visualized by vanillin-sulfuric acid, Bontrager and Dragendorff reagent. Terpene is appeared as blue spot after spray and heat by vanillin-sulfuric acid. With Dragendorff reagent alkaloid spontaneously give orange-brown spot. For Bontrager reagent, anthraquinone give red spot, anthrone give yellow color under UV 365nm and coumarins give blue spot under UV 365 nm. (Touchstone and Dobbins, 1978; Wagner and Bladt, 1996)

Results and Discussion

DPPH and ABTS radical scavenging activity

The twenty-three methanolic supernatant extracts of marine sponges displayed significant difference DPPH radical scavenging activity ($p<0.05$) (Table 1). The supernatants of nine sponges (39.13%) which showed higher scavenging activity than 90% were screened for further study. The crude extracts from nine selected sponges were prepared by extraction with three different solvents. The results showed that those crude extracts had significant difference DPPH radical scavenging activities (Table 2). The methanolic extracts almost displayed high DPPH radical scavenging activity than acetone extracts while there were almost low activity for dichloromethane extracts. Interestingly, only the dichloromethane extract from *Stylisss* sp. had the higher DPPH radical scavenging activity than acetone and methanolic extracts, respectively. While the acetone extract from *Neopetrosia* sp. “blue” had the higher DPPH radical scavenging activity than methanolic and dichloromethane extracts, respectively. Rivera & Uy (2012) also reported the effect of different solvents on the total antioxidant of five marine sponges.

The strongest DPPH radical scavenging activity was the methanolic extracts of *Hyrtios* sp.1 with IC_{50} value of 223.5 µg/ml (Table 4). There are some literatures also indicated that *Hyrtios* was the promising source for antioxidants (Utina, 2009; Longeon *et al*., 2011; Youssef *et al*., 2013). The alkaloid 6-bromo-2′-de-N-methylaplysinopsin isolated from *Hyrtios* sp. reacted strongly with DPPH at IC_{50} value of 18 µM comparable with trolox (IC_{50} 16 µM) (Utkina, 2009). The sesquiterpene aureol isolated from *Hyrtios* sp. showed the most potent antioxidant activity with an oxygen radical absorbance capacity (Longeon *et al*., 2011). Youssef *et al.* (2013) separated hyrtioerectines D–F from *Hyrtios* sp. which showed DPPH radical scavenging activity at 45, 31 and 42% respectively. The different assays, different extraction solvents and different collection localities were proved to make different antioxidant activity of *Hyrtios* sp. (Longeon *et al*., 2011; Chairman *et al*., 2012; Aktas *et al*., 2013)., In this study the acetone extracts of *Acantella* sp.1 showed DPPH radical scavenging activity at IC_{50} value of 323.3 µg/ml while the hydro-ethanolic extract of this sponge were reported with IC_{50} value of 56.94 µg/ml, and also the extract from *Xestospongia exigua* which should be the synonym of *Neopetrosia exigua* had IC_{50} at 89.17 µg/ml which lower than the methanolic extracts of this species in this study (Abdillah *et al*., 2013) so the effect of solvents on the different level of DPPH radical scavenging activity was confirmed.

The moderate DPPH radical scavenging activities were belonged to methanolic extracts of *Neopetrosia* sp. and dichloromethane extracts *Stylissa* sp. with IC_{50} values of 300.9 and 400.3 µg/ml. The dichloromethane extracts almost displayed low DPPH radical scavenging activity except from *Stylissa* sp. There were some reports on DPPH radical scavenging activity of others sponges such as *Dysidia avara*, *Aaptos aaptos, Axinella cannabina* and *Axinella damicornis* (Utkina *et al.,* 2004; Utkina, 2009; Aktas *et al*., 2013).

Concerning to DPPH and ABTS radical scavenging activity of the sponge extracts, the activity against ABTS radicals were higher than DPPH radicals. The supernatants from thirteen sponges (56.62%) showed significant ABTS radical scavenging activity higher than 90% (Table 1). The different solvents also showed significant difference ABTS radical scavenging activity (Table 3). The high activity almost found both in methanolic and acetone extracts and also lower for dichloromethane extracts except from *Stylissa* sp. and *Hyrtios* sp. 2 which theirs ABTS radical scavenging activity were quite high at 99.94±0.10 and 99.95±0.07%. Furthermore, three sponges, *Neopetrosia* sp. “blue”, *Stylisss* sp. and *Hyrtios* sp.2 had ABTS radical scavenging activity higher than 90% in all three solvent extracts.

Interestingly, the couple strongest radical scavenging activity against both DPPH and ABTS radicals presence in the methanolic extracts of *Hyrtios* sp.1. This sponge extract displayed more potent ABTS radical scavenging activity than DPPH with IC_{50} values of 77.14 µg/ml (Table 5). Some extracts also showed high ABTS radical scavenging activity such as acetone and methanolic extracts of *Neopetrosia* sp. and acetone extracts of *Hyrtios* sp.2 with IC_{50} values of 116.6, 130.5 and 181.5 µg/ml, respectively. More sponge extracts showed moderate activity against ABTS such as methanolic extract of *Neopetrosia exigua*, methanolic extract

of *Stylissa* sp., acetone extract of *Pseudoceratina* sp., acetone extract of *Acanthella* sp.1, methanolic extract of *Paratetilla bacca* and methanolic extract of *Acanthella* sp.1 with IC_{50} values of 208.3, 220.9, 218, 323.3, 337.6 and 386.1 µg/ml. There was a report on moderate ABTS radical scavenging activity of zyzzyanones A-D and makaluvamines C, E, G, H , and L isolated from the marine sponge *Zyzzya fuliginosa*. The structure-activity relationships showed that antioxidant activities of those compounds depended on the presence of a phenolic function in molecules (Utkina, 2013).

Table 1 The percentage of DPPH, ABTS radical scavenging activity and ferrous chelating activity of the supernatants from 23 Thai marine sponge at the concentration of 1 mg/ml

Sponges	Radical scavenging activity		Fe chelating
	DPPH	ABTS	
Acanthella sp.1	49.36±1.95^{e}	99.74±0.09^{a}	7.87±1.28^{kl}
Acanthella sp.2	52.67±2.76^{de}	61.38±1.33^{fg}	13.37±2.21^{ijk}
Axinyssa sp.	46.26±2.91^{efg}	78.18±2.45^{e}	54.98±5.61^{c}
Callyspongia (Cladochalina) subarmigera	66.01±1.68^{c}	81.06±2.14^{de}	31.43±2.59^{fg}
Callyspongia (Callyspongia) sp.	32.80±0.93^{h}	51.79±0.24^{h}	42.89±3.20^{de}
Callyspongia (Cladochalina) sp.	97.65±0.93^{a}	83.65±1.27^{d}	nd*
Chondrilla sp.	97.86±0.37^{a}	99.57±0.58^{a}	23.03±2.86^{ghi}
Clathria (Thalysias) reinwardti	48.50±2.91^{ef}	80.97±2.30^{de}	67.49±3.23^{b}
Coelocarteria singaporensis	27.03±1.51^{h}	30.86±3.25^{i}	nd
Hyrtios sp.1	93.27±1.70^{a}	93.27±1.47^{c}	nd
Hyrtios sp.2	71.62±2.92^{c}	98.90±0.47^{ab}	94.85±1.22a
Neopetrosia carbonaria	27.99±1.51^{h}	57.75±2.80^{g}	19. 16±1.45^{hij}
Neopetrosia exigua	98.40±0.56^{a}	99.63±0.09^{a}	10.04±1.87^{jkl}
Neopetrosia sp. *"blue"*	97.86±0.98^{a}	99.16±0.66^{a}	43.59±2.50^{de}
Neopetrosia sp.	98.18±1.30^{a}	93.85±0.55^{bc}	nd
Pachastrissa nux	41.56±1.88^{g}	64.30±3.64^{f}	31.50±6.69^{ef}
Paratetilla bacca	96.69±0.19^{a}	99.35±0.18^{a}	31.85±3.63^{fg}
Paratetilla sp.	57.26±2.43^{d}	98.84±0.24^{ab}	50.39±2.01^{cd}
Plakina sp.	41.88±3.11^{fg}	62.57±2.45^{fg}	22.56±2.15^{ghi}
Pseudoceratina sp.	97.76±0.32^{a}	99.47±0.33^{a}	27.86±1.63^{fgh}
Stelletta carvosa	84.72±4.49^{b}	99.32±0.24^{a}	15.94±1.43^{ijk}
Stylissa sp.	98.99±1.09^{a}	99.73±0.45^{a}	97.69±1.44^{a}
Xestospongia testudinaria	71.85±3.30^{c}	99.32±0.09^{a}	35.78±2.54^{ef}
Ascorbic acid	96.18±0.32	98.95±0.09	nt**
EDTA	nt	nt	87.98±0.65

The different superscript letters in each assay (in the same column) showed the significant difference from Tukey comparisons

**nd = not detectable, **nt= not tested*

More reports were confirmed that *Hyrtios* was interesting genera. Secondary metabolites of *Hyrtios* displayed diverse biological activities such as antimicrobial, free radical scavenging, cytotoxic and cancer growth inhibition activities. (Kobayashi *et al*., 1993; Youssef *et al.,* 2002; Youssef *et al.,* 2005; Youssef *et al.,* 2013). A recent study was found that *Hyrtios gumminae* collected from Andaman sea, Thailand possessed cytotoxic sesterterpenoids compound (Mahidol *et al*. 2009).

Ferrous chelating activity

Transition metals deteriorates the food and leads to the arthritis and cancer by promoting lipid peroxidation and metal chelating activity method is depends on the ability of extract to chelate transition metals. The supernatants of 23 sponges extracted with methanol were also screened by ferrous chelating activity. There were only two sponges (8.70%), *Stylissa* sp. and *Hyrtios* sp.2 approved to had ferrous chelating activity higher than 90%. *Stylissa* sp. should be considered for its potency of antioxidant activities which possessed higher than 90% for all three assays; DPPH, ABTS radical scavenging and ferrous chelating activity. When comparing the effect of different solvents, the methanolic extracts had higher ferrous chelating activity than acetone and dichloromethane, respectively. The most significant ferrous chelating activity was determined for methanolic extracts of *Stylissa* sp. and *Hyrtios* sp.2 with IC_{50} values of 159.4 and 185.6 µg/ml. A report on the ferrous chelating activity of the aqueous and methanolic extracts of marine sponge, *Geodia perarmata* showed that aqueous extract had higher ferrous chelating ability than alcoholic extract at 84.35±0.2 and 39.93±0.01%. (Taheri & Jalalinezhad, 2015)

Table 2 The percentage of DPPH radical scavenging activity of nine sponges extracted with three different solvents

Sponges	Methanol	Acetone	Dichloromethane
Callyspongia (*Cladochalina*) sp.	84.92±0.78^{de}	53.07±1.44^{hi}	58.11±3.72^{gh}
Chondrilla sp.	55.81±2.11^{gh}	43.93±0.59^{j}	21.61±1.72^{l}
Hyrtios sp.1	98.34±0.88^{abc}	61.05±1.23^{g}	30.40±0.22^{k}
Neopetrosia exigua	98.98±0.22^{abc}	70.24±1.35^{f}	17.53±0.97^{l}
Neopetrosia sp. “blue”	96.30±1.81^{abc}	100.12±0.59^{ab}	80.59±2.43^{e}
Neopetrosia sp.	100.51±0.80^{a}	17.37±2.72^{l}	35.11±0.99^{k}
Paratetilla bacca	92.08±1.93^{cd}	47.87±4.52^{ij}	15.45±7.41^{l}
Pseudoceratina sp.	91.83±0.22^{cd}	61.05±1.93^{g}	15.58±0.80^{l}
Stylissa sp.	81.48±0.96^{e}	93.21±2.58^{bc}	99.77±0.37^{ab}

The different superscript letters showed the significant difference from Tukey comparisons

Phenol and flavonoid content and others functional groups from TLC

The metabolites which are well known for thiers antioxidant activities were alkaloid, phenol , flavonoid and phenylpropanoid (Utkina *et al*., 2004; Youssef *et al*., 2013). There was a report on moderate ABTS radical scavenging activity of zyzzyanones A-D and makaluvamines C, E, G, H , and L isolated from the marine sponge *Zyzzya fuliginosa*. The structure-activity relationships showed that antioxidant activities of those compounds depended on the presence of a phenolic function in molecules. It was known that the antioxidant activity of phenolic compounds depends on the number and relative placement of hydroxyls. (Utkina, 2013)

Table 3 The percentage of ABTS radical scavenging activity of thirteen sponges extracted with three different solvents

Sponges	Methanol	Acetone	Dichloromethane
Acanthella sp.1	92.23±2.06^{bcde}	97.03±0.10^{abc}	46.09±3.09^{kl}
Chondrilla sp.	90.76±3.97^{cde}	80.60±2.75^{fg}	54.91±3.76^{ij}
Hyrtios sp.1	99.03±0.10^{a}	99.43±0.10^{a}	66.57±2.33^{h}
Hyrtios sp.2	98.40±0.40^{ab}	98.00±1.00^{ab}	99.95±0.07^{a}
Neopetrosia exigua	98.06±0.20^{ab}	93.78±1.54^{abcd}	28.06±1.89^{n}
Neopetrosia sp. “blue”	99.54±0.39^{a}	99.95±0.06^{a}	93.22±3.25^{abcd}
Neopetrosia sp.	97.94±0.45^{ab}	94.46±0.94^{abc}	60.02±2.00^{h}i
Paratetilla bacca	99.77±0.37^{a}	85.95±1.07^{ef}	74.16±4.06^{g}
Paratetilla sp.	99.54±0.10^{a}	35.81±1.82^{m}	37.16±3.29^{m}
Pseudoceratina sp.	99.54±0.10^{a}	99.66±0.00^{a}	49.62±4.25^{jk}
Stelletta carvosa	54.83±2.40^{ij}	75.33±3.12^{g}	36.55±3.34^{m}
Stylissa sp.	99.94±0.09^{a}	99.71±0.20^{a}	99.94±0.10^{a}
Xestospongia testudinaria	40.78±0.26^{lm}	87.44±2.26^{de}	39.58±2.03^{lm}

The different superscript letters showed the significant difference from Tukey comparisons

Table 4 The IC_{50} values (µg/ml) of DPPH radical scavenging activity of nine sponges extracted with three different solvents

Sponges	Methanol	Acetone	Dichloromethane
Chondrilla sp.	>1,000	>1,000	>1,000
Callyspongia (*Cladochalina*) sp.	728.2	>1,000	>1,000
Hyrtios sp.1	223.5	>1,000	>1,000
Neopetrosia exigua	608.5	>1,000	>1,000
Neopetrosia sp. “blue”	595.3	570.9	856.9
Neopetrosia sp.	300.9	>1,000	>1,000
Paratetilla bacca	645.1	>1,000	>1,000
Pseudoceratina sp.	618.3	>1,000	>1,000
Stylissa sp.	672.1	671.1	400.3

Table 5 The IC_{50} values (µg/ml) of ABTS radical scavenging activity of thirteen sponges extracted with three different solvents

Sponges	Methanol	Acetone	Dichloromethane
Acanthella sp.1	386.1	323.3	>1,000
Chondrilla sp.	452.2	>1,000	>1,000
Hyrtios sp.1	77.14	>1,000	>1,000
Hyrtios sp.2	557.5	181.5	408
Neopetrosia exigua	208.3	556.2	>1,000
Neopetrosia sp. “blue”	943	980.2	>1,000
Neopetrosia sp.	130.5	116.6	>1,000
Paratetilla bacca	337.6	632.4	>1,000
Paratetilla sp.	>1,000	>1,000	>1,000
Pseudoceratina sp.	>1,000	218	>1,000
Stelletta carvosa	>1,000	>1,000	>1,000
Stylissa sp.	2209	833.8	421.9
Xestospongia testudinaria	474.6	>1,000	>1,000

The results showed that phenol content from each solvent extracts were significant difference ($p<0.05$) (Table 6). The phenol contents were in the wide range between 3.74±0.37-124.27±3.73 mgGAE/g extract and the maximum value belonged to methanolic extract of *Hyrtios* sp.1. The highest phenol content in *Hyrtios* sp.1 was correlated to its highest DPPH and ABTS radical scavenging activity. Phenolic metabolites

such as 15-methoxypuupenol, 2-methyl-2-pentaprenyl-6-hydroxychromene and (+)-curcuohenol from marine sponge was reported as the best anti-DPPH radical cpompounds and also retarded the auto-oxidation of linseed oil (Utkina *et al*., 2004).

Hyrtios sp. has proven to be a rich source of biologically active diverse secondary metabolites of different classes. Prominent reported classes from this genus include sesquiterpene (Longeon *et al*., 2011), sesterterpenes (Qiu *et al*., 2004; Youssef *et al*., 2002; Mahidol *et al*., 2009), macrolides (Kobayashi *et al*, 1993) and alkaloids (Salmoun *et al.,* 2002; Utkina, 2009; Youssef *et al*., 2013).

The flavonoid content from each solvent extracts were also significant difference ($p<0.05$) (Table 6). The flavonoid contents were in the wide range 3.37±0.59-92.09±1.30 mgQTE/g extract and *Pseodoceratina* sp. had the maximum values. The high flavonoid content in methanolic extract of *Pseodoceratina* sp. was not correlated with its low DPPH and ABTS radical scavenging activity.

TLC chromatogram showed the presence of alkaloid as the orange spot after spray with Dragendorff reagent. Terpene were appeared as blue spot after spray and heat by vanillin-sulfuric acid. Anthrone and coumarin were appeared as yellow spot and blue spot after sprayed with Bontrager reagent under UV 365 nm. These groups were suggested to expressed the antioxidant activities. Alkaloid metabolites isolated from *Hyrtios* sp. were reported to react strongly with DPPH (Utkina, 2009; Youssef *et al*., 2013). The sesquiterpene from *Hyrtios* sp. also showed the most potent antioxidant activity (Longeon *et al*., 2011). Utkina *et al.* (2004) reported that terpene from sponge *Sarcotragus spinulosus* and *Didiscus aceratus* displayed the DPPH radical scavenging activity while Chairman *et al.* (2012) found that the active compound from extracts of *Rhabdastrella globostellata* and *Spirastrella inconstans* were phenol and alkaloid.

Table 6 The Phenol and flavonoid content of thirteen sponges extract with three different solvents

Sponges	Phenol (mgGAE/ g extract			Flavonoid (mgQTE/g extract)		
	Methanol	Acetone	Dichloromethane	Methanol	Acetone	Dichloromethane
Acanthella sp.1	21.43±0.13^{mno}	82.40±3.89^{b}	37.36±1.99^{hijk}	17.90±0.41^{lm}	182.86±3.24^{a}	25.59±1.04^{kl}
Chondrilla sp.	24.98±2.78^{lmn}	37.47±1.86^{hijk}	28.09±1.44^{klm}	31.28±0.75^{k}	95.64±1.31^{d}	48.71±0.83^{ij}
Hyrtios sp.1	124.27±3.73^{a}	119.40±3.77^{a}	67.78±3.84^{c}	26.58±0.51^{k}	131.75±5.74^{bc}	78.71±8.28^{e}
Hyrtios sp.2	49.78±3.37^{def}	43.98±1.97^{fghi}	87.16±2.67^{b}	77.69±2.81^{ef}	78.46±0.32^{ef}	69.00±1.00^{g}
Neopetrosia exigua	90.58±3.57^{b}	23.80±1.34^{lmn}	26.60±3.28^{lm}	45.98±2.63^{j}	53.00±1.00^{hij}	8.16±0.41^{no}
Neopetrosia sp. “blue”	49.78±0.21^{de}f	115.52±3.85^{a}	46.96±3.56^{efgh}	51.53±0.52^{hij}	125.08±1.74^{c}	47.69±1.57^{ij}
Neopetrosia sp.	56.07±3.83^{de}	15.65±3.00^{no}	21.07±3.40^{mno}	57.60±0.68^{h}	12.86±0.64^{mn}	14.74±0.81^{mn}
Paratetilla bacca	39.05±2.98^{ghij}	15.45±0.79^{no}	27.00±0.37^{klm}	30.94±1.00^{k}	27.90±1.04^{k}	45.08±0.49^{j}
Paratetilla sp.	11.56±0.47^{nop}	15.52±1.42^{no}	14.47±0.47^{nop}	10.21±2.12^{mno}	12.00±2.00^{mn}	12.69±2.49^{mn}
Pseudoceratina sp.	60.00±2.45^{cd}	37.40±1.52^{hijk}	21.49±1.35^{mno}	92.09±1.30^{d}	46.19±0.22^{j}	65.89±2.87^{g}
Stelletta carvosa	15.40±1.02^{no}	31.58±2.75^{jklm}	21.45±1.58^{mno}	23.29±0.15^{kl}	133.41±3.50^{b}	70.55±3.24^{fg}
Stylissa sp.	43.67±2.57^{fghi}	48.58±3.61^{efg}	23.65±4.75^{lmn}	67.09±2.66^{g}	98.46±1.63^{d}	55.00±5.00^{hi}
Xestospongia testudinaria	4.56±0.41^{p}	33.80±2.81^{ijkl}	22.65±11.63^{mn}	3.37±0.59^{o}	8.00±2.00^{no}	12.13±2.21^{mn}

The different superscript letters in phenol column or flavonoid column showed the significant difference from Tukey comparisons

Conclusion

The present study indicated the antioxidant activity of marine sponge extracts from Thailand coasts and depicted Thai marine sponges as a potential source of antioxidants. The most interesting extracts were from *Hyrtios* sp.1 and *Stylissa* sp. which had the strongest radical scavenging activity and ferrous chelating activity. The solvents used for extraction had significant effect to the scavenging and chelating activity. Therefore it can be further taken for separation and purification the bioactive compounds responsible for the antioxidant activity. More assays for antioxidant activity should be evaluated to find out more interesting antioxidants from those marine sponges. This study indicated that sponges are provides a potential sources of antioxidants that can contribute to the development of novel and effective useful pharmaceutical and industrial purposes.

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