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Antioxidant Properties of Soft Coral *Dendronephthya* sp.

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Abstract: Present study was conducted to determine the antioxidant property of soft corals belong to the genus: *Dendronephthya* (Family: Nephtheidae) using α, α-Diphenyl-β-PicrylHydrazyl (DPPH) radical-scavenging and Ferric Thiocyanate (FTC) methods using vitamin E as a positive control. Crude extracts were prepared from 4 *Dendronephthya* sp. using aqueous, dichloromethane: methanol and methanol extraction. All crude extracts of *Dendronephthya* sp. exhibited antioxidant properties and the white spots appeared during the rapid screening using Dot-Blot and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) staining where the concentration of crude extract was 1.00 g mL^{-1} against the DPPH concentration of 0.4 mM. In DPPH assay, not all crude extracts showed a significant value in antioxidant activities thus it could be considered as weak free radical species scavenger. The crude extract were diluted to 1000, 500, 250, 125, 63, 31, 15, 7, 4 and 2 μg mL^{-1}, respectively and tested against highly diluted DPPH (0.06 mM). The IC_{50} for all crude extracts were greater than 1000 μg mL^{-1}. The percentage of free radical scavenging exhibited by the crude extract was at 2 μg mL^{-1} concentration (the lowest concentration in serial dilution) with 0.81 to 2.89%. Ferric Thiocyanate (FTC) assay, showed absorbance ranges for control, vitamin E and sample were recorded as 0.012-0.858, 0.001-0.315 and 0.001-0.886, respectively. Inhibition percentage of all the crude extract was closer to the control indicated that they are weak lipid peroxidation inhibitor. However, the aqueous extract of species A and C showed higher inhibition percentage from other extract with the percentage value of 10.8 and 10.5% , respectively.

Key words: Antioxidant property, *Dendronephthya* sp., nephtheidae, radical scavenger, lipid peroxidation

INTRODUCTION

Antioxidants play an important role in the protection of human body against damage by Reactive Oxygen Species (ROS) (Govindarajan *et al*., 2005). Antioxidant system includes enzymatic and non-enzymatic components Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and catalase (CAT) are the major antioxidant enzymes and the non-enzymatic antioxidants consist of endogenous components such as uric acid, reduced glutathione and albumin) in addition to dietary antioxidants such as carotenoids, flavonoids ascorbic acid and α-tocopherol (Rietveld and Wiseman, 2003). Highly reactive free radicals and oxygen are present in biological systems from a wide variety of reactions. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. The apparent role of various antioxidant compounds in trapping free radicals makes it considerable in biomedical and pharmacological industries.

It has been well documented that the soft corals are a highly diverse group of marine organisms which are known to contain a rich variety of secondary metabolites. Despite lack of efficient physical protection in the highly competitive and hostile environment, soft corals rely on their chemical defensive system by secondary metabolites accumulating in their bodies or releasing to their surroundings for survival. The chemical defensive functions of these secondary metabolites were found to serve as antipredatory, antimicrobial, allelopathy and antifouling agents (Changyun *et al*., 2008). Among *Cnidaria*, 21% of the species contain potential marine biomedical compounds (Jha and Zi-Rong, 2004). Almost 50% of soft corals as members of *Cnidaria* have been reported to produce toxin; about 60% of their extracts contain bioactive compounds with medical properties (Higa *et al*., 2001; Sheu *et al*., 2002). Edrada *et al*. (2000) mentioned that organo-solvent extracts of soft corals are comprised mainly of lipid and sterols (90-95%) while 10% of the organic extracts are biologically active diterpenes or sesquiterpenes. The most interesting is that, many novel metabolites were isolated and their structures were elucidated (Duh *et al*., 1999; El-Gamal *et al*., 2005; Yin *et al*., 2005). For example, widely distributed soft coral

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belong to the genus *Sinularia* produces toxin. It has been reported that about 60% of *Sinularia* corals from 73 species produce/contain toxin including sesquiterpenes, diterpenes, norditerpenes, polyhydroxylated steroids and polyamine compounds with antimicrobial, anti-inflammatory and cytotoxic activities (Khalesi *et al*., 2007). In addition, over 210 research studies have been published about the chemical compounds of *Sinularia*, from which the majority report novel cytotoxic terpenoids (Khalesi *et al*., 2007).

Although many bioactive compounds of soft coral were isolated, there are very few published studies in their antioxidant activity. Zhang *et al.* (2005) found a new hemiketal steroid, named cladiellin A and its derivatives, first isolated from the soft coral *Cladiella* sp. Bioassay showed that these compounds have antioxidant property. Recently, two new bioactive sesquiterpenes were isolated from ethyl acetate soluble portion of soft coral *Sinularia* sp., Both compounds showed antioxidant activities (Zhang *et al*., 2006).

Since there were no study had been carried out to investigate the antioxidant properties of *Dendronephthya* sp., this study was aimed to explore the antioxidant potential of 4 species belong to the genus *Dendronephthya.*

MATERIALS AND METHODS

Collection and identification of soft coral: Samples were collected from Pulau Payar, Langkawi by Self Contained Underwater Breathing Apparatus (SCUBA) diving during December 2008. The fresh samples were stored at 0°C temporarily during sampling to prevent any chemical degradation. The samples were then identified to the genus level (Ellis and Sharron 1997; Benayahu *et al*., 2004) prior to deep freezing at-20°C until extraction (Satyajit, 2006; Bhakuni and Rawat, 2005).

EXTRACT PREPARATION

Aqueous extraction: Two hundred gram of samples were added with 500 mL distilled water and powdered stored at 0°C in cold room for 24 h. The samples were then thawed and centrifuged to separate residue and water extract. The centrifuged samples were filtered and the residues/filtrate were collected for organic solvent extraction. The aqueous extract were frozen in the deep freezer (-20°C) and freeze-dried to remove water.

Dichloromethane: Methanol extraction: The residue from aqueous extraction were soaked with 500 mL of DCM: Methanol solvent (250: 250 mL, v/v). The DCM: Methanol-soaked samples were mixed well using orbital shaker for 24 h at room temperature. After 24 h, the soaked samples were filtered and the residues/filtrate were collected for methanol extraction. The solvents were evaporated using rotary evaporator to obtain concentrated extract.

Methanol extraction: The residues from DCM: MeOH extraction were added with 250 mL methanol and mixed well for 24 h at room temperature. After 24 h, the samples were filtered and the extracts were evaporated.

Rapid screening of antioxidant using dot-blot and DPPH staining: Rapid screening of antioxidant using the method Dot-Blot and DPPH staining was adopted with slight modification (Soler-Rivas *et al*., 2000). The crude extracts were dissolved with their solvent (distilled water, methanol: Dichloromethane and methanol). The extracts and vitamin C were carefully loaded on TLC layer and dried for 3 min. The 0.4 mM DPPH solution was sprayed onto the TLC layer. The stained TLC layer revealed a purple background with white spot at the location of the drops which showed radical scavenger capacity.

Free radical scavenging assay: The free radical scavenging activities of different extracts were assessed by following the method described by Banerjee *et al.* (2008), Shimoda *et al.* (1992) and Nsimba *et al.* (2008). The stock solution of each extract was dissolved in methanol and 1 mg mL^{-1} concentration was prepared. From the stock solution, the serial dilution was performed in triplicate (500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 1.95 μg mL^{-1} concentration). Each extract (100 μL) was mixed with 3.9 mL of freshly prepared solution containing 25 mg L^{-1} of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in methanol. The absorbance was measured at 517 nm after 30 min by UV spectrophotometer. The percentage of DPPH scavenging activity is calculated as follows:

$$\text{Scavenging activity (\%)} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

A lower absorbance indicates a higher scavenging effect. EC_{50} value (mg mL^{-1}) is the effective concentration at which DPPH radicals were scavenged by 50%. Vitamin C and E were used as positive control.

Ferric thiocyanate (FTC) method: The FTC method was described by Huang *et al.* (2005) and Aqil *et al.* (2006) was adopted in this experiment with slight modification. Four milligram crude extract was added to the assay mixture. The percent inhibition of linoleic acid peroxidation was calculated as:

Table 1: The IC_{50} and percent of DPPH inhibition at 2 μg mL^{-1} of different soft coral crude extracts

Crude extract	IC_{50} (μg mL^{-1})	Percentage inhibition at 2 μg mL^{-1}
Water A	<1000	2.29±0.4
Water B	<1000	0.81±0.2
Water C	<1000	2.12±0.2
Water D	<1000	2.42±0.5
DCM:MeOH A	<1000	1.18±0.2
DCM:MeOH B	<1000	1.36±1.2
DCM:MeOH C	<1000	2.26±0.1
DCM:MeOH D	<1000	2.89±0.1
MeOH A	<1000	2.56±0.5
MeOH B	<1000	1.29±0.5
MeOH C	<1000	2.00±1.1
MeOH D	<1000	1.64±0.1
Vit C	<125	4.17±1.1
Vit E	<250	3.50±1.8

A = Species A, B = Species B, C = Species C, D = Species D, Water = Aqueous extract: DCM:MeOH = Dichoromethane:Methanol extract; MeOH = Methanol extract, Vit C= Vitamin C, Vit E = Vitamin E. Data represent Mean±SD of three independent experiments performed in triplicate

Table 2: Results of antioxidant assay for crude extracts from *Dendronephtya* sp. by FTC method

	Samples													
Absorbance	Control	Water A	Water B	Water C	Water D	DCM: MeOH A	DCM: MeOH B	DCM: MeOH C	DCM: MeOH D	MeOH A	MeOH B	MeOH C	MeOH D	Vit E (nm)
d-0	0.012	0.003	0.006	0.001	0.010	0.007	0.003	0.001	0.003	0.005	0.011	0.006	0.005	0.001
d-1	0.189	0.182	0.187	0.178	0.183	0.189	0.190	0.187	0.185	0.190	0.190	0.182	0.199	0.066
d-2	0.224	0.202	0.214	0.203	0.212	0.224	0.222	0.219	0.213	0.229	0.228	0.227	0.242	0.074
d-3	0.309	0.289	0.299	0.294	0.284	0.300	0.302	0.302	0.299	0.310	0.295	0.308	0.313	0.103
d-4	0.321	0.309	0.319	0.311	0.314	0.334	0.321	0.311	0.320	0.338	0.323	0.327	0.336	0.112
d-5	0.486	0.453	0.470	0.456	0.460	0.479	0.478	0.466	0.467	0.486	0.480	0.485	0.497	0.162
d-6	0.858	0.831	0.865	0.852	0.860	0.886	0.864	0.846	0.862	0.868	0.858	0.868	0.875	0.286
d-7	0.528	0.471	0.494	0.472	0.486	0.496	0.492	0.508	0.496	0.516	0.518	0.511	0.524	0.315

Absorbance reading until day-7 (one day after control reaching maximum)

$$\text{Inhibition (\%)} = 100 - \frac{\text{Absorbance increase of sample}}{\text{Absorbance increase of control}} \times 100$$

All tests were run in triplicate and vitamin E was used as a positive control.

RESULTS AND DISCUSSION

Rapid screening of antioxidants using dot-blot and DPPH staining: This method was based on the inhibition of the accumulation of oxidized compounds and the generation of free radicals that is inhibited by the addition of antioxidant. All crude extracts developed white spot in Dot-Blot assay indicated the presence of antioxidants. The intensity of the white/yellow colour depends on the amount and nature of radical-scavenger present in the extract. Thus, all the crude extract contains the antioxidant compound (Huang *et al*., 2005; Chang *et al*., 2007; Walter *et al*., 2003; Jianchun *et al*., 2006; Yakovleva *et al*., 2004).

DPPH (1,1- diphenyl-2-Picrylhydrazyl) assay: No crude extract of *Dendronephthya* sp. showed strong antioxidant activity with IC_{50} below than 1000 μg mL^{-1} concentration in DPPH assay. Hence all the extracts were of weak free radical scavenger with IC_{50}<1000 μg mL^{-1} (Table 1). This might be due to some factors could complicate or hinder the bioactivity of the crude extract hence the quantity of bioactive metabolites can't be measured in the crude extract. The presence of responsible active compound for antioxidant activities in trace amount might require more crude extract from huge amount of *Dendronephtya* sp. On the other hand the instability of the metabolites needs to be overlooked. It has been suggested that marine extract may contain extremely labile compounds. Decomposition of these compounds might occur at any step during extraction. Heat, light, air and pH are among the factors that might lead to the degradation of compounds. Besides, the abundance of salts in crude extract carried over from sea water could make the bioassay inaccurate (Bhakuni and Rawat, 2005; Sharma and Bhat, 2009; Alma *et al*., 2003; Karioti *et al*., 2004; Kordali *et al*., 2005).

Ferric Thiocyanate (FTC) assay: The antioxidant effects of *Dendronephthya* sp. extract and vitamin E on the peroxidation of linoleic acid were investigated and the results are represented in Table 2. The absorbance range recorded for control, vitamin E and samples were 0.012-0.858, 0.001-0.315 and 0.001-0.886, respectively. Compared to the control and positive control (vitamin E), no extract of *Dendronephthya* sp. produced higher inhibition of lipid peroxidation. Inhibition percentage of all the crude extracts was closer to the control which

Table 3: The absorbance values and percentage of linoleic acid peroxidation by the crude extracts of *Dendronephtya* sp. as measured by FTC antioxidant assay

Sample	Absorbance*	Percentage of inhibition
Control	0.528	0
Water A	0.471	10.8
Water B	0.494	6.5
Water C	0.472	10.5
Water D	0.486	7.9
DCM:MeOH A	0.496	6.1
DCM:MeOH B	0.492	6.7
DCM:MeOH C	0.508	3.7
DCM:MeOH D	0.496	6.1
MeOH A	0.516	2.2
MeOH B	0.518	1.8
MeOH C	0.511	3.2
MeOH D	0.524	0.7
Vit E	0.315	40.3

A = Species A, B = Species B, C = Species C, D = Species D, Water = Aqueous extract, DCM:MeOH = Dichoromethane:Methanol extract, MeOH = Methanol extract, Vit, E = Vitamin E. *Absorbance reading (until day-7) (one day after control reaching maximum)

indicated that they are of weak lipid peroxidation inhibitor. However, the aqueous extract of species A and C showed higher inhibition compare to other extracts with inhibition percentage of 10.8 and 10.5%, respectively (Table 3).

In this method, the linoleic acid was reduced by Fe^{2+} to free radical, while the ferrous ion itself undergone oxidation process to Fe^{3+}. Then, the Fe^{3+} ion reacted with thiocyanate ion $(SCN)^-$ to give complex $Fe(SCN)_3$ with a bright red color. The low absorbance values in this experiment were corresponding to a high percent of inhibition thus revealed that sample could inhibit lipid peroxidation and hence having considerable antioxidant property.

CONCLUSION

Antioxidants are considered important nutraceuticals on account of many health benefits (Droge, 2002; Lee *et al*., 2004; Valko *et al*., 2007). Inhibition percentage crude extracts indicated that they are of weak lipid peroxidation inhibitor. All the extracts of *Dendronephthya* sp. were of weak free radical scavenger with IC_{50}<1000 μg mL^{-1}. Hence The requirement of a standard assay is very important in order to compare the results of different laboratories and validation of the conclusions.

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