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Nutritional and Antioxidant Properties of Sea Slug (*Discodoris* sp.) from Pamekasan Indonesia Sea Water

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Abstract

Sea slug, *Discodoris* sp., is an important organism for Pamekasan people, as it is used for nutraceutical and functional food. However there is lack comprehensive scientific study regarding the efficacy of *Discodoris* sp. in improving people health. Therefore an experiment was conducted to study the nutritional and bioactive compound of *Discodoris* sp from Pamekasan Indonesia sea water. *Discodoris* sp was collected from coastal wetland and mangrove swamps of Pamekasan. Sample was cleaned, dried and mashed. The proximate, amino acids, fatty acids and antioxidant compound were evaluated following the accepted procedure. *Discodoris* sp has a potential as nutraceutical and functional food as it contained high protein. Fatty acid content of *Discodoris* sp. was dominated by unsaturated fatty acids with linolenic acid (C18:3, n-3) as the highest component. *Discodoris* sp. contained high level of alkaloid, steroid and phenolic compound which posseses antioxidant properties. *Discodoris* sp contained high nutrient and beneficial for human health.

Keywords: *Discodoris* sp., phytochemical, Pamekasan, antioxidant, alkaloid

1. Introduction

Indonesian marine ecosystem is rich with natural resource and biodiversity and appears as “epicentre” of marine tropical biodiversity (Veron 1995). Highly variation in type of island and archipelago are the reasons for this large biodiversity (Gray 1997). *Discodoris* sp is a type of nudibranchs live in coastal and mangrove swamps. Hutomo & Moosa (2005) stated that this invertebrate is commonly found in

mudflat coastal ecosystem as epibenthic organism. *Discodoris* sp. has been used empirically by coastal people of Pamekasan Madura as food and drugs to cure breast ulcer in breastfeeding women. The organism is also used as traditional herb to heal backache and as aphrodisiacs. At a scientific study, *Discodoris* sp powder reduced LDL level and increased HDL level in rabbit blood serum (Nurjanah 2009).

Nudibranchs possessed secondary metabolites derived from the sponge they prey on and this metabolites act as allelochemicals to protect against predators (Miyamoto 2006). Fahey& Garson (2002) found halogenated metabolits from extracts of the dorid nudibranch *Asteronotus cespitosus*. Nudibranch *Tambja eliora* possesses tambjamine D which diplays cytotoxic properties in V79 Chinese hamster lung fibroblast cells (Cavalcanti et al 2008). Some researches has also been done on *Discodoris* sp. Ibrahim (2001) has isolated steroid compound that contained androgenic function. Witjaksono (2005) stated that *Discodoris* sp contained saturated and unsaturated fatty acid and sterol on non-polar fraction. Phytochemistry test shown *Discodoris* sp. from Buton Island Indonesia contained some chemical substance played a role as antioxidant (Nurjanah et al. 2010). However there is a lack comprehensive experiment on nutritional value and bioactive components on *Discodoris* sp. Therefore, an experiment was conducted to study the nutritional value and bioactive compound of *Discodoris* sp from Pamekasan Indonesia sea water.

2. Materials and Methods

2.1. Proximate, Amino Acids, Fatty Acids and Mineral Analysis of *Discodoris* sp.

Discodoris sp. was taken from coastal water of Pamekasan Madura. The sample was cleaned and separated from the viscera then sun-dried for 3-4 days and mashed. Sample of fresh and dried *Discodoris* sp.were analysed in triplicates for proximate composition following to method of AOAC (2005).

2.2. Determination of Amino Acid

Amino acids content was determined through HPLC (Varian 940-LC) method. The amino acid sample was prepared by adding 5 mL 6 M HCl onto 0.2 g homogenate. The sample was dried at 100 °C for 24 h, and then filtered. The filtrate was mixed with methanol, sodium acetate and triethylamine solution (ratio 2:2:1) and evaporated with nitrogen gas. Dried sample was derived using methanol, thiocianic acid, and triethylamine solution (ratio 3:3:4) and dissolved in 10 ml 60% acetonitril and keep for 20 minutes. Amino acid composition was analyzed using HPLC and the sample was filtrated prior injected into HPLC. The analysis was run at 27 °C with 1 ml/minutes flow rate and 3000 psi pressure. Acetonitril 60% and phosphate buffer 0.1 M were used as mobile phase and the absorbance was detected at 256 nm.

2.3. Determination of Fatty Acid

Fatty acid was determined by GC-MS (Agilent Technologies). Fatty acid was extracted with soxhlet method and derivatived according to AACC method (1983). The condition of GC-MS for fatty acid analysis consisted 200 °C of coloum temperature with initial temperature at 150 °C and final temperature at 180 °C. Temperature increase rate was programmed at 5 °C/minutes. Nitrogen was used as carrier gas and flow rate 2,5 Kgf/cm^3 (H_2) and 50 Kgf/cm^3 (N_2). Fatty acid was calculated as percentage of total lipid.

2.4. Determination of Mineral

Mineral composition were determined by means atomic absorption spectroscopy (AAS Shimazu-7000).

2.5. Extraction of Antioxidant Compound from *Discodoris* sp.

Extraction of antioxidant compound from *Discodoris* sp. was carried out through solvent extraction followed method of Sherif et al. (2008) with some modifications. Three different solvents with gradual polarity were used on bioactive compound extraction, i.e. chloroform (non polar), ethyl acetate (semi polar) and ethanol (polar). *Discodoris* sp. (50 g) was crushed and soaked in 100 mL of chloroform for 24 hours at room temperature. The mixture was then filtered through Whatman no 1 filter paper and the residue was extracted with ethyl acetate and ethanol respectively in the same way. All of three solutions were evaporated and dried under vacuum (below 40 °C), to yield the chloroform, ethyl acetate, and methanol extracts, respectively.

2.6. Determination of Phytochemical Component

Secondary metabolite component of *Discodoris* sp extract was qualitatively determined using common phytochemical test (Harborne 1973).

2.7. Determination of Antioxidant Activity

Antioxidant acitivity was measured according to method of Blois (1958) with slight modification. A 500 µl of a 1.0 mM DPPH methanol solution was added to a solution of the extract or standard (4.5 ml) and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity (AA %). Solution of BHT served as positive control. Percent scavenging of the DPPH free radical was measured using the following equation:

$$\%\ Inhibition = \frac{Ac - As}{Ac} \times 100\%$$

Where:
Ab: Absorbance of control
As: Absorbance of sample solution

3. Result and Discussion

Discodoris sp. is a brown blackish shellless mollusc from nudibranch order live in muddy rock or sandy region at coastal environment. The proximate composition of meat and viscera of *Discodoris* sp. from coastal region of Pamekasan Madura are shown in Table 1. Meat and viscera of *Discodoris* sp. contain high protein (12.31-13.60%) and low fat (0.44%). Protein content of *Discodoris* sp. from Pamekasan was lower than *Discodoris* sp. from Belitung or Buton Island (Andriyanti, 2009; Nurjanah, 2010) as well as with *Discodoris boholensis* (Witjaksono, 2005), while the ash and carbohydrate content were higher. The differences could be affected by ecological properties, such season and nutrition factor (Hawkins, 1985). Comparing with freshwater molluscs *Mytilus galloprovincialis* Lmk. (Fuentes et al., 2009), *Discodoris* sp. contained higher in protein content, but lower in ash and fat. However, it was lower compared to giant land snail (Fagbuaro, 2006), mussels (*Mytilus galloprovincialis*, L.) (Sengor et al., 2008) and adductor of giant clam *Tridacna gigas* (Southgate, 1996)

Table 1: Proximate composition of *Discodoris* sp.

Proximate composition (dry weight)	Current Study		Discodoris boholensis [(a)]	Discodoris sp.[(b)]
	Meat	Viscera		
Moisture	11,17±1,49	9,22±1,11	19,36	15,25
Ash	17,96±0,25	26,86±0,64	10,69	11,74
Protein	45,13±0,29	37,57±1,21	59,79	49,60
Fat	2,67±0,14	7,10±0,44	5,84	4,58
Carbohydrate	23,07±1,83	19,26±0,86	4,42	18,83

(a); Witjaksono (2005), (b); Nurjanah (2010)

Amino acids compositions of *Discodoris* sp. are presented in Table 2. *Discodoris* sp contain essential and nonessential amino acids, which dominated with leucine and glutamic acid, respectively. The essential amino acid of the meat and viscera of *Discodoris* sp was ranged from 0.28-1.42 and 0.25-1.67 mg/100 mg of the dry weight, respectively. Meanwhile, the total of nonessential amino acid in meat and viscera was 6.54 and 6.76 mg/100 mg of the dry weight, respectively. Leucine and lysine represented 50.6% and 50.7% of the essential amino acid of meat and viscera of *Discodoris* sp, respectively. Glutamate and proline represented 51.5% and 49.2% of the nonessential amino acid of *Discodoris* sp, respectively. Glutamate was the most abundant non essential amino acid, similar result were found in *Archatina archatina,* and *Archachatina marginata* giant land snail (Adeyeye and Afolabi 2004), mussel (*Mytilus galloprovincialis*, L.) (Sengor et al., 2008) and giant clam *Tridacna gigas* (Southgate, 1996).

Table 2: Amino acids composition of *Discodoris* sp. in mg/100mg of dry weight

Amino Acids	Meat	Viscera
Leucine	1,42	1,67
Lysine	1,40	1,22
Valine	0,81	0,83
Threonine	0,52	0,57
Isoleucine	0,43	0,34
Phenylalanine	0,36	0,46
Histidine	0,35	0,35
Methionine	0,28	0,25
Glutamatic Acid	2,19	2,14
Proline	1,18	1,19
Aspartic Acid	0,91	0,96
Serine	0,55	0,61
Tyrosine	0,50	0,48
Arginine	0,46	0,52
Alanine	0,39	0,44
Glysine	0,22	0,26
Cysteine	0,14	0,16
Essential Amino Acid	5,57	5,69
Nonessential amino acid	6,54	6,76

Discodoris sp. contained C12, C14 and C16 as saturated fatty acid and ω3, ω6, and ω9 as unsaturated fatty acid (Table 3). Unsaturated fatty acid content was higher than saturated fatty acid in meat of *Discodoris* sp. however it was lower in *Discodoris* sp. viscera. Ekin and Bashan (2010) found unsaturated fatty acid were higher than saturated fatty acid in all tissue of *Unio elongatulus*. Similar result also found by several researchers (Erson and Sereflisan 2010; Shanmugam et al., 2007; Murphy et al., 2003). Linolenic acid and palmitate was dominated in meat, presented 60.3% of the unsaturated fatty acid, and 48.5% of the saturated fatty acid, respectively. Linolenic acid are important component of membrane cell and has beneficial effect in health and control of chronic diseases (Simopuolos, 1999)

Table 3: Fatty acid profile of *Discodoris* sp.

Fatty Acid	Meat (%)	Viscera (%)
C16:0	13,36	16,74
C12:0	4,58	3,53
C14:0	1,11	2,68
C18:0	8,48	6,87
Saturated Fatty Acid	27,53	29,82
18:1 n9	8,12	7,63

Table 3: Fatty acid profile of *Discodoris* sp. - continued

18:3 n3	20,91	4,22
18:2 n3	5,63	6,10
Unsaturated Fatty Acid	34,66	17,95

Mineral composition of *Discodoris* sp. are presented in Table 4. Three macro elements (K, Ca and Mg) and seven micro elements (Fe, Zn, Mn, Pb, Cd, Hg and As) were determined by atomic absorption spectroscopy from meat and viscera of *Discodoris* sp. The highest macro element was potassium (206.05 ppm), while magnesium was the lowest macro element found in *Discodoris* sp. both meat and viscera. Such interesting phenomenon was occurred on trace element. The meat contained high Zn and low Mn, meanwhile the viscera occurred the opposite condition. Others trace element such as lead, cadmium, mercury and arsenic were detected at low-to-none concentration. It is indicated that *Discodoris* sp. was not contaminated with dangerous heavy metal. Fagbuaro et al. (2006) reported the calcium and potassium were among the highest mineral found in the flesh of four species of giant snail, while zinc, and manganese were detected at low concentration. Similar result were also found in mussel, flying squid, octopus and squid (Karakoltsidis et al., 1995). Some other molluscs contained high level of sodium (Fuantes et al., 2009; Astorga-España et al., 2007).

Table 4: Mineral compositon of *Discodoris* sp.

Mineral composition	Meat (ppm)	Viscera (ppm)
K	197,86	206,05
Ca	179,98	187,66
Mg	110,95	112,40
Zn	7,52	4,35
Fe	6,99	5,17
Mn	6,93	7,25
Pb	0,73	0,59
Cd	ND	ND
Hg	ND	ND
As	ND	ND

Potassium and calcium play important roles in human health. Potassium could lowers blood pressure, reduce the risk of stroke, prevent renal vascular, glomerulal and tubular damage, reduces urinary calcium excretion and reduces ventricular arhyhmias (He FJ and MacGregor GA., 2001). Meanwhile calcium uptake beneficial for bone health (Ilich and Kerstetter., 2000), lower systolic blood preasure among overweight children whose mothers were suplemented with calcium (Belizan et al., 1997)

Meat and viscera powder of *Discodoris* sp were extracted to obtained bioactive compound by solvent extraction method with three different solvent. Qualitative analysis result of bioactive compound on *Discodoris* sp. extract were presented in Table 5. From three different solvents, *Discodoris* sp. extract contained alkaloid, and steroid in meat and viscera. Saponin was found only in ethanol-extracted meat, while flavanoid was found only in etanol-extracted viscera.

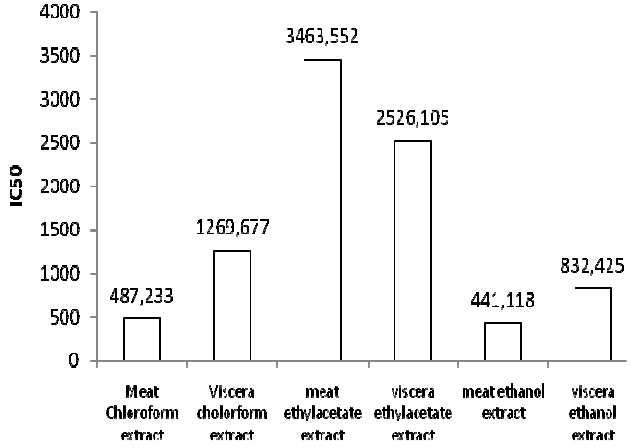
Table 5: Phytochemical analysis of *Discodoris* sp extract

Phytochemistry	Meat Extract			Viscera extract		
	Chloroform	Ethyl Acetate	Ethanol	Chloroform	Ethyl Acetate	Ethanol
Alkaloid:						
Meyer	+++	++	+++	+++	+++	+++
Wagner	+++	+	+++	++	+++	+++
Dragendrof	+++	++	+++	+++	+++	+++
Steroid	+	++	+++	+	++	+
Flavanoid	-	-	-	-	-	+
Saponin	-	-	++	-	-	-
Phenol hydroquinone	-	-	++	++	+++	-

Alkaloid is secondary metabolites produced by a large variety of organism and the most abundant substance in higher plant and has biological activity for human (Aniszewski, 2007). Class of nudibranch contained variety of alkaloid which possessed biological activity. Nudibranch is known have defensive ability through production secondary metabolite which give toxic or antifeedant effect for it predator and some of these chemical defense are obtained from dietary source (Haber et al., 2010; Avila 2000; Derby 2007).

Extract of *Discodoris* sp has a IC_{50} value range from 441-3633 µg/mL (Fig 1). The highest antioxidant value was obtained from ethanol-extracted meat. The value is higher compared with *Spirulina maxima* extract (Miranda et al., 1998), however it still lower than commercial antioxidant BHT i.e 397,04 ppm. Marine organism is known has antioxidant activity in response to prevent the negative effect of reactive oxygen species (ROS) which may react in an aggressive manner, destroying cellular compartments, tissues and, finally, organisms and populations. Antioxidant activity has positive correlation with xenobiotic contamination, reproductive seasonality, food availability and ontogenetic development (Filho et al., 2001). Antioxidant is beneficial for biological system through several mechanisms: terminating radical chain reaction, chelating transition metal, reducing agent and stimulating antioxidative enzyme (Gorinstein et al., 2003).

Figure 1: Antioxidant activity of *Discodoris* sp extract against DPPH



4. Conclusion

Discodoris sp contained high nutrient and beneficial for human health. *Discodoris* sp contain high protein and low fat. The mollusc also contained antioxidant compound.

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References

[1] American Association of Cereal Chemist [AACC]. 1983. *Approved Methods of The American Association of Cereal Chemist.* Ed ke-8. Maret. USA :American Association of Cereal Chemist.

[2] Adeyeye EI, and Afolabi EO. 2004. Amino acid composition of three different types of land snails consumed in Nigeria. Food Chemistry 85: 535–539.

[3] Andriyani, R. 2009. Extraction of antioxidant compound of sea slug (*Discodoris sp.*) from Belitung Island. Undergraduate Thesis. Bogor. Bogor Agricultural University.

[4] Aniszewski, T. 2007. *Alkaloids - secrets of life*. Elsevier: Amsterdam, 335 pp.
[5] AOAC. 2005. *Official Methods of Analysis* (18 ed). Association of Official Analytical Chemist Inc. Mayland. USA.
[6] Astorga-España, M.S., Rodríguez-Rodríguez, E.M. and Díaz-Romero, C. 2007. Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile), *Journal of Food Composition and Analysis* **20** (3-4): 273–279.
[7] Avila, C., Iken, K., Fontana, A. and Cimino, G. 2000. Chemical ecology of the Antarctic nudibranch *Bathydoris hodgsoni* Eliot, 1907: defensive role and origin of its natural products. Journal of Experimental Marine Biology and Ecology 252 :27-44.
[8] Belizan, J.M., Villar, J., Bergel, E., del Pino, A., Di Fulvio, S., Galliano, S.V. and Kattan, C. 1997. Long-term effect of calcium supplementation during pregnancy on the blood pressure of offspring: follow up of a randomised controlled trial. BMJ 315:281–5.
[9] Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181: 1199-1200.
[10] Cavalcanti, B.C., Júnior, H.V.N., Seleghim, M.H.R., Berlinck, R.G.S., Cunha, G.M.A., Moraes, MO. and Pessoa, C. 2008 Cytotoxic and genotoxic effects of tambjamine D, an alkaloid isolated from the nudibranch *Tambja eliora*, on Chinese hamster lung fibroblasts. Chemico-Biological Interactions. 174(3): 155-162.
[11] Derby, C.D. 2007. Escape by Inking and Secreting: Marine Molluscs Avoid Predators Through a Rich Array of Chemicals and Mechanisms. Biol. Bull. 213: 274–289
[12] Ekin, I. and Bashan, M. 2010. Fatty Acid Composition of Selected Tissues of *Unio elongatulus* (Bourguignat, 1860) (Mollusca: Bivalvia) Collected from Tigris River, Turkey. Turkish Journal of Fisheries and Aquatic Sciences 10: 445-451
[13] Erson, B. and Sereflisan, H. 2010. The Proximate Composition and Fatty Acid Profiles of Edible Parts of Two Freshwater Mussels. Turkish Journal of Fisheries and Aquatic Sciences 10: 71-74
[14] Fagbuaro, O., Oso, JA., Edward, J.B. and Ogunleye, R.F. 2006. Nutritional status of four species of giant land snails in Nigeria. Journal of Zhejiang University SCIENCE B 7(9):686-689.
[15] Fahey, S.J. and Garson, M.J. 2002. Geographic Variation of Natural Products of Tropical Nudibranch *Asteronotus cespitosus.* Journal of Chemical Ecology 28(9): 1773-1785.
[16] Filho, W.S., Tribess, T., Gaspari, C., Claudio, F.D., Torres, M.A., and Magalhaes, A.R.M. 2001. Seasonal changes in antioxidant defences of the digestive gland of the brown mussel (*Perna perna*). Aquaculture 203:149–158
[17] Fuentesa, A., Fernández-Segovia, I., Escrichea, I. and Serra, J.A. 2009. Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. Food Chemistry 112(2): 295-302
[18] Gorinstein, S., Moncheva, S., Katrich, E., Toledo, F., Arancibia, P., Goshev, I. and Trakhtenberg S. 2003. Antioxidants in the black mussel (*Mytilus galloprovincialis*) as an indicator of Black Sea coastal pollution. Mar Pollut Bull 46:1317–1325
[19] Gray, J.S. 1997. Marine biodiversity: patterns, threats and conservation needs. Biodiversity and Conservation 6: 153-175
[20] Haber, M., Cerfeda, S. and Carbone M, et al. 2010. Coloration and Defense in the Nudibranch Gastropod *Hypselodoris fontandraui*. Biol. Bull 218: 181–188.
[21] Hanani, E., Mun'im, A. and Sekarini, R. 2005. Identification of antioxidant compound on Sponge *Callyspongia* sp. from Kepualauan Seribu. Majalah Ilmu Kefarmasian, 2(3):127 –133.
[22] Hawkins, A.J.S. 1985. Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis L.* Oecologia 66(1): 42-49.
[23] He, F.A. and MacGregor, G.A. 2001. Fortnightly Review: Beneficial effects of potassium. BMJ. 2001;323:497–501

[24] Houghton, P.J. and Raman, A. 1998. *Laboratory Handbook for the Fractionation of Natural Extracts*. Chapman and Hall, London.
[25] Hutomo, M. and Moosa, M.K. 2005. Indonesian marine and coastal biodiversity: Present status. Indian Journal of Marine Sciences 34(1): 88-97.
[26] Ibrahim, M. 2001. Isolation and biological activity test of steroid compound of sea slug *Discodoris* sp. M.Sc. thesis. Bogor: Bogor Agricultural University.
[27] Ilich, J. and Kerstetter, J.E. 2000. Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr 19:715–37
[28] Karakoltsidis, P.A., Zotos, A. and Constantinides, S.M. 1995. Composition of the commercially important mediterranean finfish, crustaceans, and molluscs, Journal of Food Composition and Analysis **8**: 258–273
[29] Miranda, M.S., Cintra, R.G., Barros, S.B.M. and Mancini-Filho, J. 1998. Antioxidant activity of the microalga *Spirulina maxima*. Braz J Med Biol Res 31(8): 1075-1079.
[30] Miyamoto, T. 2006. Selected Bioactive Compounds from Japanese Anaspideans and Nudibranchs. Pp. 199-214. *In*: Climino G, and Gavagnin M. (eds). Molluscs: From Chemo-ecological Study to Biotechnological Application. Springer. Berlin.
[31] Murphy, K.J., Mann, N.J. and Sinclair AJ. 2003. Fatty acid and sterol composition of frozen and freeze-dried New Zealand Green Lipped Mussel (Perna canaliculus) from three sites in New Zealand. Asia Pacific J Clin Nutr 12(1): 50-60.
[32] Nurjanah, Hardjito, L., Monintja, D.R., Bintang, M. and Agungpriyono, D.R. 2009. Anticholesterolemic of Sea Slug (*Discodoris* sp) in Rabbit New Zealend White. Jurnal Kelautan Nasional 2: 31-42.
[33] Rohman, A., Riyanto, R. and Diah, U. 2006. Aktivitas antioksidan, kandungan fenolik total dan kandungan flavonoid total ekstrak etil asetat buah Mengkudu serta fraksi-fraksinya. Majalah Farmasi Indonesia. 17(3):136–142.
[34] Sengor, G.L., Gun, H. and Kalafatoglu, H. 2008. Determination of the Amino Acid and Chemical Composition of Canned Smoked Mussels (*Mytilus galloprovincialis*, L.) Turk. J. Vet. Anim. Sci. 32(1): 1-5
[35] Shanmugam, A., Palpandi, C. and Sambavisam, S. 2007. Some valuable fatty acids exposed from wedge clam *Donax cuneatus* (Linnaeus). African Journal of Biochemistry Research 1(2): 014-018.
[36] Ebada, S.S., Edrada, R.A., Lin, W. and Procksch, P. 2008. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrata. Nature Protocols 3(12):1820-1831.
[37] Harborne, J.B., 1973. Phytochemcial methods. London, Chapman and Hall, Ltd., pp: 49-188.
[38] Simopuolos, A.P. 1999. Essential fatty acids in health and chronic disease. American Journal of Clinical Nutrition 70(3): 560S-569S
[39] Southgate, P.S. 1996. The chemical composition of giant clam (*Tridacna gigas)* Tissue. Asian Fisheries Science 9: 143-148
[40] Veron, J.E.N. 1995. Corals in Time and Space. Sydney: University of New South Wales Press
[41] Witjaksono, H.T. 2005. Chemical composition of extract and oil of sea slug (*Discodoris boholensis*).MSc. Thesis. Bogor: Bogor Agricultural University.