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Research Article

Steroid Content of Some Species of Deep Sea Fish in Western Sumatra and Southern Java Ocean

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Abstract: In this research, some researchers suspect steroid hormone content on *Bathypteorois* and *Erimoensis* species of *Bajacalifornia atricolor*. The purpose of this research is to know steroid content. This research used the 11 species of marine fish in the Western ocean obtained in Southern Sumatra and Java through research vessel Baruna Jaya IV, in September and October 2004. The species are *Alepocephalus bicolor*, *Antigonia rubescens*, *Barbourisia rufa*, *Bajacalifornia eromoensis*, *Caelorinchus divergens*, *Polymixia* sp, *Rouleina guentheri*, *Setarches guentheri*, *Synagrops japonicus*, *Tydermania dalgleishi* and *Xenolepidichthys*. From the results of proximate analysis, the nutrient content in some marine fish are 11.94-20.81% protein contents, 0.01-4.84% fat contents, 73.29-82.73% moisture contents and 1.07-2.49% ash content. On analysis of HPLC (High Performance Liquid Chromatography) the fat contents of steroid obtained are between 0026-0.016%. Deep sea fish species that assumed have steroids compounds are *Bajacalifornia eriomoensis*, *Caelorinchus divergens*, *Tydermania navigatoris*, *Roulenia guentheri*, *Antigonia rubencens*, *Alepocephalus bicolor* and *Synagrops japonicus*. *Bajacalifornia erimoensis* showed the best results on HPLC test so it continued with infrared test to show the similarity of steroid group.

Keywords: Deep sea fish, steroid, Western Sumatra and Southern Java Ocean

INTRODUCTION

The Department of Marine and Fisheries mentions that during the 2004-2009 period there was an increase in fisheries production by an average of 225,24% in 2009 and is expected to reach 5,37 million tons for capture fisheries and 3.25 million tons for aquaculture (Salim, 2010). Utilization of marine fisheries in Indonesia mostly from the pelagic fisheries with depth ranges between 0-100 m. The increasing of fishing every year in fishing ground caused overfishing. This case can make fish stock over lack. To expect this thing, then developing the new fishing ground is need with searching potential in marine fish resources in Indonesian water.

In the expedition of Baruna Jaya IV research vessel which did research in the Indian Ocean start from Southern of Java Ocean until Western Sumatra, found 530 species of marine fish, 70 species were recently identified and the scientific name is not yet known. The team also found the known fish species, it was *Bathypteorois erimoensis* and *Bajacalifornia atricolor* (Suman *et al*., 2006).

The current efforts of new drug fulfillment are filled through the exploratory work that search by variety of the structure of drug compound that clinically still in use and exploit natural resources. One of resources that have been not developed maximally is natural water resources such deep sea water. Deep-sea is part of the environment nautical located under depth that can be lighted sunlight in the open sea and deeper than the continental slope (>200 m) (Nybakken, 1992). Broad deep waters in indonesia reach 40% of total indonesian waters (Pasaribu, 2003).

Bioactive compounds have been found in various marine organisms such as algae, sponge and palisade. These bioactive compounds were secondary metabolites that produced from living organism self-defense when against the predator (Fanany, 2005). One of these bioactive compounds as steroid that assumed will find in deep-sea fish. These compounds used as vitality supplement and stamina (Jamaludin, 2005). Deep sea fish that not yet known its benefits, need to doing researches to find out the benefits of deep-sea fish as one of raw materials in fisheries, especially in Indonesia. It also adds information about deep-sea fish, especially about steroid compounds as preliminary information.

MATERIALS AND METHODS

The main material that used is marine fish in the Southern Sumatra and Western Java Ocean. Other materials used in this research is the chemical for

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solvent testing such as methanol, ethyl acetate and chloroform and kjeltab tablet, solution of H2SO4, NaOH, CH3COOH, H3BO4, HCL, methyl red indicator, fat solvent hexane, ice and fat-free cotton.

Research procedure: Raw materials obtained from BRPL (Marine Fisheries Research Agency) in Muara Baru, North Jakarta. Preparation the necessary tools and the place of research is the first step. Then, proximate test carried out for the study of next stage of the research include of extraction steroid compounds, Liebermann-Burchard test, TLC, HPLC test and FTIR test. As the first stage of research conducted an analysis of sea fish samples proximate in further tested of steroid hormone content. Analysis includes the level of protein, fat, carbohydrate, water and ash.

Steroid content presence analyzed by Liebermann-Burchard method, with few drop of anhydrous acetic acid and addition of 0.5 mL chloroform at least extract deep-sea fish and stirred. Then add one drop of sulphuric acid. The green color emergence indicated that the extracts contain steroid. Steroid extraction as bioactive compound has done before and continuously with steroid hormone presence test.

RESULTS AND DISCUSSION

Proximate analysis: Table 1 showed the proximate analysis of deep-see fish about moisture composition, ash, fat, protein and carbohydrates. Proximate contents on some type of deep-sea fish have different content. The differences caused by environmental differences among others, temperature, salinity, pressure, oxygen availability and more. Deep sea fish need fat to keep body temperature because of the environment in the deep ocean has a lower temperature than pelagic environment/surface (Davis, 1991). When compared with tuna, some deep-sea fish have higher fat content. It is because deep-sea fish movement is less than the pelagic fish (Nybakken, 1988).

Moisture content: Moisture content in deep-sea fish in contained in two forms: free water and bound water. Free water content found in the spaces between cells and plasma that can dissolve a wide range of vitamins, mineral salts and certain nitrogen compounds. Bound water found with chemically bounding, pshycochemical bounding and cappilary bounding.

Deep-sea fish contained higher water that tuna. It caused by having high salinity, more than 34.2%. When compared with tuna, deep-sea fish has higher water than tuna (pelagic fish), according to Nybakken (1992), the water in body tissues in deep-sea fish has increased with increasing depth.

Ash content: Some type of deep sea fish have higher ash content than pelagic fish. It caused by different mineral content from different species of fish.

Fat content: Fat content of some species of deep-sea fish affected by several factors, such as species, the catching season, geographical location, level of gonad maturity and the size of the fish (Kusumo, 1997).

Deep sea condition that has extreme temperature under 10°C about 3-5% caused deep-sea fishes adapted with feeding so these fish have high fat content. Low temperature of fish body also caused low metabolism. Consequently the movement and their growth also slow, a little reproduce and longer than the animals on warm water environment (Davis, 1991).

Protein content: Deep-sea fish have higher protein content than tuna. It caused by the differences of zone where the food of mesopelagyc have no sunshine so that there are no phytoplankton which is one source of protein that consumed by the deep sea (Davis, 1991). Nybakken (1992), wrote that the higher level of depth of a living organism, the less feed is available. This is because the potential ingredients for animal feed, which sank in the sea has been used by organisms in the top layer of the deep ocean. The least amount of feed intake caused the nutrients in deep-sea fish especially protein is relatively low.

Extraction of bioactive compound: Extraction of bioactive compounds in deep-sea fish is done by using the Quin method (1988) in Fanany (2005). The solvent that used were chloroform, ethyl acetic and water. Amount of 40 g samples of deep-sea fish crushed by mortar. Volume solvent that used is about 120 mL.

Table 2 may indicate that chloroform solvent extract then ethyl acetate at same ranges 50%. Steroid

Table 1: Proximate analysis on deep-sea fish

Species	Water content	Ash content	Lipid content	Protein content	Carbohydrate content
Caelorinchus divergens	80.75	1.03	0.01	18.14	0.07
Antigonia rubescens	74.27	1.77	0.02	19.82	4.12
Alepocephalus bicolor	74.50	1.23	4.84	14.92	4.51
Barbourisia rufa	82.58	1.07	2.87	11.94	1.54
Xenolepidichthys dalgleishi	76.65	1.39	0.84	19.55	1.57
Rouleina guentheri	82.73	1.41	2.39	12.75	0.72
Tydermania navigatoris	75.27	2.48	2.58	18.34	1.33
Synagrops japonicus	73.29	1.42	1.35	20.58	3.36
Polymixia sp	74.77	1.32	0.73	20.81	2.37
Setarches guentheri	78.35	1.17	0.02	19.60	0.86
Bajacalifornia erimoensis	83.49	0.83	2.40	11.75	1.53
Pelagic fish (tuna)	70.58	1.30	1.01	22.00	0.00

Table 2: Extraction rendemen result

Species	Sample weight	Chloroform		Acetate ethyl	
Alepocephalus bicolor	40.76	1.96	4.85	0.45	1.11
Antigonia rubescens	40.80	0.25	0.61	0.20	0.49
Barbourisia rufa	40.06	7.20	17.97	1.63	4.07
Caelorinchus divergens	40.35	0.56	1.39	0.86	2.13
Polymixia sp	40.25	0.38	0.94	1.27	3.16
Rouleina guentheri	40.32	0.39	0.97	1.32	3.27
Setarches guentheri	40.83	0.10	0.24	0.14	0.34
Synagrops japonicus	40.67	0.39	0.97	2.00	4.92
Tydermania navigatoris	40.46	0.89	2.20	1.46	3.61

Table 3: *Liebermann-Burchard*

No	Species	Results
1	*Bajacalifornia erimoensis*	++
2	*Barbourisia rufa*	-
3	*Caelorinchus divergens*	+
4	*Xenolepidichthys dalgleishi*	-
5	*Tydermania navigatoris*	+
6	*Rouleina guentheri*	+
7	*Polymixia* sp	-
8	*Antigonia rubescens*	++
9	*Setarches guentheri*	++
10	*Alepocephalus bicolor*	+
11	*Synagrops japonicus*	+
12	Pelagic fish (tuna)	-

Description: +: Positive result; -: Negative results

compounds are soluble in non polar solvents. Chloroform is a non polar solvent so extract chloroform used for identification of steroids. Deep sea fish extraction results can be affected by several factors, such as the natural condition of materials, an extraction method that used, the particle size of the sample and the prolonged storage condition (Herry, 2005).

Test Liebermann-Burchard: Colourtest give the result test with color change to the presence of steroid hormone qualitatively. It showed that several types of deep sea fish have a positive result that indicated by the presence of the green color on the test result. These results can determine the presence of the steroid nucleus in phytochemicals.

Table 3 indicates that some species of deep sea fish have more value than pelagic fish because of steroid compound. The species that have steroid compound are *Bajacalifornia erimoensis*, *Caelorinchus divergens*, *Tydermania navigatoris*, *Rouleina guentheri*, *Antigonia rubescens*, *Setarches guentheri*, *Synagrops japonicas*. The discoloration that occurs is from blue to green. These colors came from tryterpenoid which is a cyclic structure compound, mostly in the form of alcohol, aldehyde, or carboxylic acids (Harbone, 1987).

TLC test (thin layer chromatography): Testing of TLC is done to deep-sea fish that had positive result in *Liebermann-Burchard* test. The test showed that the patches formed on silica gel by using wave UV light 254 nm, giving positive results on the seven fish. When viewed from Rf score of the fishes then seven fishes have same RF score with standard. It is more convincing that in *Bajacalifornia eromoensis*, *Caelorinchus divergens*, *Tydermania navigatoris*, *Roulenia guentheri*, *Antigonia rubencens*, *Alepocephalus bicolor* and *Synagrops japonicas*, *Alepocephalus bicolor*, have the presence of steroid compound.

HPLC Test (High Performance Liquid Chromatography): The largest steroid compounds found in *Bajacalifornia erimeonsis* with 25, 76 mg/g and the smallest in *Synagrops japonicus* with 16.44 mg/g. Steroids are derived from fat which is one of the main components of fat. Range of sterol derivatives for example cholesterol, bile acids and steroids (Piliang *et al*., 2006).

Infrared test: Infrared spectro is used to see the chemical structure through infrared rays. Infrared provides information about functional group type that is contained in steroids. Testing of FT-IR is taken 2 samples of *Bajacalifornia japonicus* and *Synagrops erimoensis* as a sample to find out the characteristics of steroid compounds more simply. Infrared test on *Bajacalifornia erimoensis* showed some of the top which is the core of the steroid bond.

From amount of C atom on *Bajacalifornia erimoensis* assumed that it is steroid group with C atom between C_{27}-C_{30}. O-H bonds in 3.200-3.600 possition is hydrocarbon, where all the steroid molecule is a derivative of trisyclyc aromatic hydrocarbons (Fessenden and Fessenden, 1982). The C-O bond at 1.150 tied to rings D steroid molecule usually on C-17 chains (Dence, 1980). The A ring and B ring did not fused with the aldehyde group/cetones then steroid compounds that contained in *Bajacalifornia erimoensis* are reactive. Steroid compound did not have benzene ring, benzene ring on the steroid A structure is steroid molecule type of estrogen.

C-H bound in 2.850-2.950 possitions showed saturated metyl. Steroid compound in *Synagrops japonicus* is thought to be one of the estrogens demonstrated by the presence of moieties aromatic at the 1.490-1.620 positions (Fessenden and Fessenden, 1982). Steroid compound in *Synagrops japonicus* have at least 28 C atoms. C28 is includes of the steroid (Bahti *et al*., 1985). *Bajacalifornia japonicus* and

Synagrops erimoensis provide the difference in a chemical bond that is similar to the steroid standard. These can provide different types of steroid compound contained on these species.

CONCLUSION

Chemical composition of deep-sea fish proximate analysis are 73.29-82.73% moisture content, 1.07-2.49% ash content, 0.01-4.84% fat content, and 11.94-20.81% protein. Yield result showed 17.97-0.61% in chloroform and 4.92-0.34% in ethyl acetate.

Liebermann-Burchard test showed that some species of deep sea fish have positive results against the presence of steroids and steroid compounds. From TLC test positive results obtained Rf.

HPLC test showed the existence of steroids, 25.76 mg/g in *Bajacalifornia erimoensis*, 22.43 mg/g in *Caelorinchus divergens*, 20.21 mg/g in *Tydermania navigatoris*, 18.90 mg/g in *Rouleina guentheri*, 18.30 in mg/g *Antigonia rubescens*, 16.94 mg/g in *Alepocephalus bicolor* and 16.44 mg/g in *Synagrops japonicus*.

Infrared test results on *Bajacalifornia erimoensis* showed some of steroid compound with reactive chemical bonds type.

REFERENCES

Bahti, H.H., T.J. Rukmiati and A.D. Yeyeh, 1985. Isolation and Identification of Steroid Compound and Related Compound of Alkaloid from Frangipani Trees Leaves (*Plumiera Acutifolia Poir*). [Research Project], Mathematic and Science Faculty, Padjajaran University, Bandung.

Davis, R.A., 1991. Oceanography, An Introduction to the Marine Environment Ed ke-2. Wm. C. Brown Publishers, USA.

Dence, J.B., 1980. Steroids and Peptides. A John Wiley and Sons Inc., New York.

Fanany, F.A., 2005. Initial study of nutrient content and antibacterial from deep-sea fish in south java water. Thesis, Fisheries and Marine Science Faculty, Bogor Agricultural University, Bogor.

Fessenden, R.J. and J.S. Fessenden, 1982. Organic Chemistry. 3rd Edn., Chapter Two. Erlangga Publisher, Jakarta.

Harbone, J.B., 1987. Method of Guiding the Modern Way of Photochemical, Plants Analyze. 2nd Edn., ITB Press, Bandung.

Herry, S. 2005. Chemistry extraction operation technic. Thesis, Engineering Faculty, Diponegoro University, Semarang.

Jamaludin, D., 2005. Early study of steroid content and antibacterial activity test deep-sea fish (*Satyrichthys welchii*) of South Java water. Thesis, Fisheries and Marine Science Faculty, Bogor Agricultural University, Bogor.

Kusumo, 1997. The diversity of the fatty acids of several pelagic and demersal fish that landed in pelabuhanratu, west java and muara angke, Jakarta. Thesis, Fisheries and Marine Science Faculty, Bogor Agricultural University, Bogor.

Nybakken, J.W., 1988. Marine Biology: An Ecological Approach. Gramedia Publisher, Jakarta.

Nybakken, J.W., 1992. Sea Biology. Scholastic Main Library, Jakarta.

Pasaribu, B.P., 2003. Necessary Scientific Consortium for Deep Sea Exploration. Retrieved form: http//: www. Kompas.com, (Accessed on: 17 December 2012)

Piliang, W., G. Soewaondo and D. Al Haj, 2006. Nutrition Physiology. 1st Edn., IPB Press, Bogor, Vol. 1.

Salim, M., 2010. Maritime policy and fisheries dynamics of rembang on the reform and regional autonomy in 1998-2008. MA Thesis, Graduate School of Diponegoro University, Semarang.

Suman, A., F. Satria and E. Rahmat, 2006. Preliminary Research on the Composition of the Types, Distribution and Densities of Demersal Fish Stocks of Marine Resources in the Waters of ZEEI the Indian Ocean South of Java and West Sumatra. Fisheries and Marine Research Center, Jakarta.