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Vitamin E Potential in Extract of Anchovy *Engraulis encrasicolus* through the Increase on Gene Expression of Adiponectin in Diabetes Mellitus

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Abstract

Anchovy Engraulis encrasicolus is one of the marine resources that incredibly abundance in Indonesian waters, particularly in Southeast Province. Fish oil or Omega-3 (n-3 PUFA) in anchovy fish has essential roles for the protection of degenerative diseases. This study supposed that *anchovy* has certain micronutrients including vitamin E that has beneficial roles for the absorption of Omega-3, and provides a potential resource at the future as constituent materials for natural medicines or functional food through the increase of gene expression of adiponectin that helps blood glucose regulation for diabetes mellitus (DM) patients. For that reason, the specific aim of this study was to measure and assess vitamin E content in oil extract of *Anchovy* derived from Kendari Sea Waters, Southeast Sulawesi Province-Indonesia. Extraction of *Anchovy* samples used the Soxhlet method, whereas analysis of vitamin E used the (UV)/Vis *spectrophotometric* method. Results of the study revealed that 5 gr fresh *Engraulis encrasicolus* *Anchovy* resulted in 3 ml fish oil, whereas oil extract of *Engraulis encrasicolus* Anchovy contains (31,50 µg/g).

Keywords: Anchovy Engraulis encrasicolus; Extract;Vitamin E; Adiponectin.

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1. Introduction

Anchovy Engraulis encrasicolus that contains Omega-3 has been shown improved spatial memory in the hippocampus (an area deep of the forebrain that helps regulate memory and cognitive learning) in the treated mice *(Sprague dawley)* compared to the control group [1]. Another study reported that consumption of *Anchovy* has significantly increased adiponectin level ($p < 0.026$) and reduction of leptin level ($p < 0.024$) in patients with *systemic lupus erythematosus* (SLE) compared to baseline values after 120 days, and suggested to reinforce the importance of evaluating prospective studies concerning the consumption of fish and fish oil in an attempt to reduce degenerative disease risk factors [2]. Adiponectin is secreted by fatty tissue or adipose tissue that has anti-inflammation effects and vital roles in fat metabolism and the enhancement of insulin sensitivity [3]. Vitamin E intake modulates adiponectin expression and increases insulin sensitivity [4], inhibits production and the release of TNF-α, TGF-β1 and IL-1β in diabetic mice induced by STZ [5]. Insulin resistance and type 2 diabetes mellitus have been treated with thiazolidinediones (TZD) since 1990s, and one mechanism by which these drugs may work is through PPARγ-mediated upregulation of adiponectin, an endogenous adipokine that has been shown to increase insulin sensitivity. Interestingly, α-and γ-tocopherols, two Vitamin E, have structural similarities to the TZD and also have been linked to enhanced insulin sensitivity [6]. On the contrary, reduction of adiponectin levels in the circulation of obese people resulted in negative effects on the function of β cells and this is supposed directly linked with dysfunction of β cells in type 2 diabetes mellitus patients [7]. To the best of authors knowledge, no published studies have been conducted pertaining to the beneficial effects of Anchovy oil extract (*Engraulis encrasicolus)* on gene expression of adiponectin in diabetes mellitus patients. In view of that, the present study explores the utilization of Anchovy oil extract through the increase of gene expression of adiponectin in diabetes mellitus patients. We regard this sea biota offers a potential resource at the future utilized as constituent materials for natural medicines or food supplement or functional food derived from marine resource. Anchovy are very abundance in Southeast Province waters and it is supposed this sea biota has quality content of vitamin E for the absorption of n-3 PUFA, and for that reason, we are concerned to conduct a preliminary study by exploring this sea biota as a local wisdom potential resource.

2. Materials and Method

2.1. Sampling location

Anchovy Engraulis encrasicolus were collected from the Kendari Sea Waters, Southeast Sulawesi Province of Indonesia. Fresh samples of Anchovy were collected and sorted in the morning, and they were put into the plastic containers (Figure 1) and were placed in thermos bottles/cooling containers filled before with flaked ice. Frost condition was maintained during transportation of fresh samples of *Anchovy* from Kendari city until being processed at the Laboratory of Faculty of Pharmacy, Halu Oleo University, Kendari. The study was approved by the Ethics Committee for Health Studies, Halu Oleo University, Kendari in Southeast Province-Indonesia with the recommendation of ethical clearance stated in no. 978/UN29.20/PPM/2019.

Figure 1: Anchovy Engraulis encrasicolus collected from the Kendari Sea Waters, Southeast Sulawesi Province, Indonesia.

2.2. Preparations of Anchovy Extracts

Extraction process of *Anchovy Engraulis encrasicolus* used the soxhlet method that referred to the previous study [8] and we have extensively modified that previous method. For details, 2 kg fresh samples of *Anchovy* fish were cleaned from unwanted materials and were filleted and dried using the Froilabo oven for 24 hours at 40°C that resulted in 761.3 gr dried weight of *Anchovy*. Afterward, 5 gr *Anchovy* sample were evaporated by using 500 ml *n-hexane* solvent with evaporation process (the soxhlet) for 3 hours until drops of extracts were discolor and resulted in 0.62 g fish oil. Results of the extraction were then evaporated using the vacum rotary evaporator at 45°C that resulted in 3 ml purified extract without *n-hexane* solvent.

2.3. Measurement of Vitamin E Content

Analysis of vitamin E levels used (UV)/Vis *Spectrophotometric* that referred to the Indonesian National Standard for food and beverage testing methods (SNI 0-2891-1992). The steps of measurement of vitamin E content were beginning from the determination of calibration curve in which 4 ml of 100 mg/L alpha-tocopherol solution (1ppm =1 mg/1000ml) was put into cuvette to measure its absorbance value using UV/Vis spectrophotometer with a maximum wavelength of 294 nm. After then, serial concentrations of 20, 40, 80, 100 ppm were made to determine their regression linear ($y = ax + b$). Afterward, absorbance values for serial concentrations (20, 40, 80, 100) ppm were measured using UV/Vis spectrophotometer at a 294 nm wavelength (20 ppm = 0.002; 40 ppm = 0.080; 80 ppm = 0.275; 100 ppm = 0.361). Each concentration absorbance value was then inserted into a linear curve using the linear regression equation of $y = 0.004x – 0.09$. Subsequently, the absorbance value of 4 ml extract Anchovy *Engraulis encrasicolus* was measured (0.036) dan inserted into the linear regression equation of ($y = 0.004x – 0.09$) to measure vitamin E level in extract of *Anchovy Engraulis encrasicolus* that resulted in vitamin (E 31,5 μg/g)

3. Results

Yield of oil extract of *Anchovy Engraulis encrasicolus* was 12.4% by using the following formula:

$$\text{Yield of Fish Oil Extract} = \frac{\text{weight of fish oil (g)}}{\text{weight of fish sample (g)}} \text{ x } 100\ \%$$

$$= \frac{0.62\text{ g}}{5\text{ g}} \text{ x } 100\ \%$$

= 12.4 %

Evaporation process was done to separate *n-hexane* solvent from oil in the *Anchovy* samples by using the *vaccum rotary evaporator* at 45°C until resulted in 3 ml oil extract *Engraulis encrasicolus.*

Table 1. Vitamin E content in oil extract of *Anchovy Engraulis encrasicolus* using the UV/Vis *Spectrophotometric* method.

Table 1: Nutrient content of oil extract of *Anchovy Engraulis encrasicolus.*

No	Nutrient	Unit	Content per g	Method
1.	Vitamin E	μg	31,5	UV/Vis *Spectrophotometric*

4. Discussion

One of the beneficial effects associated with vitamin E intake is the enhancement of upregulation of adiponectin expression [9], through a mechanism that involves PPARgamma together with its endogenous ligand of15-deoxy-Delta12,14-prostaglandin J2. Comprehensive mechanism of induction of adiponectin is not already well identified, but it is considered as the basis of beneficial effects of vitamin E on insulin sensitivity [4]. Recent advances pertaining to the understanding of adiponectin have investigated the roles of adipose tissues as an endocrine active organ. One of the central regulators of adipocyte biology is peroxisome proliferator-activated receptor gamma (PPARγ), a transcriptional factor that induces adipogenic gene expression [10]. The experiment in diabetic mice revealed that diabetes induces oxidized-low-density lipoproteins (Ox-LDL) mediated oxidative stress and vascular smooth muscle cell (VSMC) proliferation in aorta of rat, are important independent cardiovascular risk factors that have been shown to stimulate vascular smooth muscle cell (VSMC) proliferation, but rats treated with vitamin E for 42 days significantly resulted in the improvement effect on reduction of vascular smooth muscle cell (VSMC) proliferation and Ox-LDL mediated oxidative stress and c-reactive protein (CRP) was also restored and imply that vitamin E has a strong protective effect as an antioxidant [11]. Several previous studies have shown that vitamin E has many more important molecular properties, with consequent prevention of oxidative damage associated with many diseases, or the modulation of signal transduction and gene expression [12]. High content of vitamin E in *Diadema setosum* can increase adaptive antibody, mainly immunoglobulin G (IgG) and gene expression FOXP3 as the source of immune regulatory in experimental animals [13,14]. Normal need of vitamin E is in the equivalent range of 10-12 mg per day and theoritically requirement of vitamin E will increase when intake of PUFA is high with a ratio of 0.4 mg/ α-tocopherol/gram of PUFA intake is considered as the reasonable vitamin E intake (Mann dan Truswell, 2012). Vitamin E is also needed as transporter of n-3 PUFA to the body tissues [15]. Interestingly, in this present study, oil extract of *Anchovy Engraulis encrasicolus* contains (31,5 μg/g) vitamin E compared to *Anchovy Engraulis ringens* (25 μg/g), *on vitamin E outcomes* [16]. Although we did not identify EPA and DHA values in oil of *Anchovy Engraulis encrasicolus*, this present study provides a new insight that vitamin E in oil *Anchovy Engraulis encrasicolus* has strong protection on the oxidation of n-3PUFA, mainly EPA and DHA in fish oil. In addition, *Anchovy* oil is commonly consumed wholly or partially as a suplement in various processed foods such as, mayonaise, yogurt and salad sauce. Consumption of *Anchovy Engraulis encrasicolus* provides good effects for health condition. Results of the study conducted by Suárez-Jiménez and his colleagues reported that oxidation of lipid in sea biota oil lead to reduction of food quality and free radicals due to oxidation of polyunsaturated fatty acid (PUFA) that generates oxidative stress [17]. This proves that vitamin E has essential roles for the stabilization of unsaturated fatty acid in lipoprotein and cell membranes as well as protection from easily oxidized compounds such as PUFA (polyunsaturated fatty acid), DNA and RNA [18,19]. Scientific innovation through the extraction of *Anchovy* oil *Engraulis encrasicolus* derived from Southeast Province waters provides a potential resource to increase gene expression of adiponectin for the regulation of blood glucose or insulin sensitivity in diabetes mellitus patients, and accordingly future studies are needed either in *in vivo* or *in vitro.* Fish oil derived from marine resource that increases adinopectin level is proportional with the increase of HDL level [2]. Anchovy *Engraulis encrasicolus* has potential roles as anti-thrombosis, anti-inflammation, enhancement of HDL level and reduction of LDL [20]. Anchovy *Engraulis encrasicolus* is important to consume because it contains fish oil or omega-3, mainly EPA and DHA that usefull for health [21, 22, 23]. Serum adiponectin level can increase HDL (high density lipoprotein) level and reduce plasma triglyceride and LDL (low density lipoprotein), accordingly adiponectin has an important role for the prevention of some metabolic diseases [24].

5. Conclusion

Anchovy Engraulis encrasicolus can be utilized as a prospective food resource for health at the future concerning this sea biota has beneficial roles on the prevention of various metabolic impairments that calls for extensive studies of n-3PUFA content, particularly EPA and DHA.

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