Effect of extraction method of the eel fish albumin levels of *Anguilla marmorata* (Q.) gaimard and *Anguilla bicolor*

Jamaluddin¹, Hasnawati¹, Y Yuyun¹, Pitriani² and A Widodo¹

¹Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Palu, Central Sulawesi, Indonesia
²Department of Environmental Health, Public Health Faculty, Tadulako University, Palu, Central Sulawesi, Indonesia

*Email: jamal_farmasi02@yahoo.co.id*

**Abstract.** This study aims to determine the effect of the extraction method based on the type and weight of the sample to the albumin levels, which can produce the highest albumin levels. The extraction method used is heating (hotplate) at heat temperature 60°C for 30 minutes using 0,1 M HCl solvent and steam (water bath) at heat temperature of 60°C (the sample temperature is 45°C) for 30 minutes with distilled water solvent. The weight of the sample used is 0,5 kg and 0,7 kg. Testing of the albumin levels using the *Bromocresol green* (BCG) method by photometer with a wavelength (λ) 546 nm. According to statistical data the highest albumin levels contained in the extraction of steaming water bath with a value of 0,156 g/100 g. Based on the type of eel, *Anguilla marmorata* (Q.) Gaimard with the highest albumin levels of 0,143 g/100 g. The highest albumin levels of the *Anguilla marmorata* (Q.) Gaimard on the weight of 0,5 kg and 0,7 kg are 0,132 g/100 g and 0,153 g/100g.

### 1. Introduction

Eel fish is a fishery commodity that has important economic worth in an international trade market especially to export purposes, therefore the utilization rate tends to increase every years [1]. Currently there are 22 species of Eel fish found in the world and 9 species are found in Indonesia, namely *Anguilla borneensis*, *A.nebulosa*, *A. cebesensis*, *A. bicolor pacifica*, *A. megastoma*, *A. interioris*, *A. obicura*, *A. bicolor bicolor*, and *A. mormorata* [2]. Distribution of eel fish in Indonesia throughout the length coast of Sumatera, south Java, Bali, West Nusa Tenggara, East Nusa Tenggara, Kalimantan, waters of Sulawesi, Maluku to Papua [3].

The existence of Eel fish in Sulawesi waters is found in several regions, namely the waters of Gorontalo, North Sulawesi, West Sulawesi, Southeast Sulawesi and Central Sulawesi [4]. In Central Sulawesi, the presence of Eel fish is abundant, especially in the Poso region, this condition supported by the deep Tomini Bay and extensive inland waters, namely Lake Poso [5].

A study conducted by [6] shows the result of fresh eel fish (*Anguilla bicolor*) chemical analysis per 100g as follows: water level of 43.03%, ash level of 6.78%, 48.80% of fat, 30.50% of protein, 16.44% of carbohydrate, and 0.04% of raw fabric. The following explanations are several extraction methods used in analyzing the albumin levels of eel fish in which, in turn, will be used to analyze the albumin levels of eel fish types *Anguilla marmorata* (Q.) Gaimard, and *Anguilla bicolor*.

In a research conducted by [7], eel fish sterilization at the temperature of 121°C for 25 minutes resulted in 116,419 of albumin filtrate level in 22,660 mg/100 g. [8] states that 15 minutes of heating at 50°C temperature and extracted using chloride acid solvent (HCl) resulted in 1.5 g/dL of albumin filtrate level. While [9] states that 12.5 minutes of heating at 35°C temperature using a vacuum extractor resulted in 2,62 g/dL of albumin level and 23.26% of ash.
A research conducted by [10] results in 2.495 g/100 g of eel fish albumin level after being heated for 23-35 minutes in 60°C of temperature by extraction by steaming using distilled water. Moreover, [11] states that the best quality of eel fish albumin powder is obtained at 49°C temperature vacuum drying with albumin levels by 4.71%. Further research by [10] states that the highest level of eel fish albumin of 1.77 mg/100 g is acquired by 30 minutes of 40°C heating through isolating the albumin-based on the characteristics of the molecule’s mass resulted from steaming extraction.

2. Materials and methods

2.1. Materials

The main substances in this research were eel fish type *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* originated from Lake Poso. The substances used to test the level of albumin using the bromocresol green method were reagen bromocresol green, albumin standard reagen, and control reagen. The other substances were distilled water, chloride acid (HCl), 0.1 M, hexane (C₆H₁₄), concentrated nitrate acid (HNO₃), 105 of sodium hydroxide (NaOH), 0.2% of sulfate copper (II) (CuSO₄).

The instruments of the research were Photometer 5010, hotplate, thermometer, porcelain cup, the stand and clamp, rod, tube rack, blender (Kirin®), analytical balance, measuring cups, a beaker, a water bath, tweezers, funnel, volumetric flask, a pipette, knife, stopwatch.

2.2. Methods

2.2.1. Samples preparation. Eel fish (*Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor*) were cleaned (removed the scales, gills, and entrails), then washed until no more blood and mucus, cut into small pieces, and bones removed. The flesh is taken then washed thoroughly and drain well, then stored in a refrigerator [12].

Preparation for heating extraction (hotplate), samples were smoothed using a blender and add distilled water solvent ratio of 1: 1 v / b (100 mL of distilled solvent: 100 g sample) of the crude extract [13; 14].

Preparation for steaming extraction (water bath), samples were cleaned (removed the scales, gills, and entrails), then washed until no more blood and mucus, cut into small pieces, and bones removed. Then, the samples were smoothed using a blender [10; 15].

2.2.2. Extraction by heating (Hotplate). First, add 50 mL of HCl 0,1 M to the prepared samples, then heated for 30 minutes at 60°C temperature. Second, separate the filtrate and residue by filtering then add 200 mL of hexane and shake for 30 minutes to form two phases, the oil phase and water phase (extract), and separate the oil phase using a funnel to the container. Last, the obtained extract (water phase) was ready to be analyzed [16].

2.2.3. Extraction by steaming (water bath). First, add 150 mL of distilled water solvent to the prepared samples, then steamed for 30 minutes at 60°C temperature (45°C meat temperature). Second, separate the filtrate and residue by filtering. Last, the obtained extract was ready to be analyzed [10].

2.3. Qualitative analysis

2.3.1. Biuret test [17]. First, prepare four sterile test tubes and fill each of them with 2 mL extract of *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor*. Second, add to each tube 1 mL of 10% NaOH and three drips of 0.2% CuSO₄. Last, observe any occurring changes, change to violet indicates the existence of albumin.
2.3.2. Xanthoprotein test [17]. First, prepare four sterile test tubes and fill each of them with 2 mL extract of Anguilla marmorata (Q.) Gaimard and Anguilla bicolor. Second, add to each tube 1 mL of concentrated HNO₃ which eventually formed a white deposit. Third, the tubes were heated for one minute until it changed to yellow and cooled under faucet water. Last, add 10% NaOH drip by drip through the wall of the tube until the layers were formed.

2.3.3. Visual test [17]. First, prepare four sterile porcelain cups and fill each of them with 5 mL extract of Anguilla marmorata (Q.) Gaimard and Anguilla bicolor were then heated in the water bath for 30 minutes at 70°C temperature. Then, observe for any occurring changes. The positive result indicated by the occurrence of a white cloud on the top of the sample extract.

2.4. Quantitative analysis

2.4.1. Albumin level test. First, albumin extract as much as 10 mL centrifuged for 10 minutes. Second, pipette 1 mL Bromocresol green solution into a test tube. Last, add 10 µL (0,1 mL) for standards, control (distilled water), and the test sample (test), then homogenized and measured the absorbance at 546 nm wavelength.

This test was used for all methods of extraction. Wherein triplo was conducted in each sample of each of the extraction methods.

2.4.2 Data analysis. Data analysis techniques used in this study were statistics analysis of F test and t-test Anova table as a comparative test of average value using SPSS 16.0 (Statistical Product and Service Solution).

3. Results and discussion

Qualitative test results: Biuret testing using 10% NaOH + 0.2% CuSO₄ reagent indicates the existence of albumin (positive), Xanthoprotein testing using a reagent and then heated + HNO₃ pa 10% NaOH indicates the existence of albumin (positive), and visual testing heated at a temperature of 70°C for 30 minutes indicates the existence of albumin (positive).

Based on the above qualitative test results, the samples are concluded positively for albumin because all of the samples contain albumin so then succeeded by the quantitative testing method of Bromocresol green (BCG) using a photometer with a wavelength (λ) 546 nm.

Quantitative test results in Table 1 shows the average value of the albumin level of heating extraction methods (hotplate), i.e. Anguilla marmorata (Q.) Gaimard (0,5kg) 0,77g / L, Anguilla marmorata (Q.) Gaimard (0,7kg) 0,77g / L, Anguilla bicolor (0,5kg) 0,40g / L, Anguilla bicolor (0.7) 0,83g / L. In table 2, the average value of albumin from steam extraction methods (water bath), i.e. Anguilla marmorata (Q.) Gaimard (0,5kg) 1,00g / L, Anguilla marmorata (Q.) Gaimard (0,7kg) 1,27g / L, Anguilla bicolor (0,5kg) 0,83g / L, Anguilla bicolor (0.7) 1,07g / L.

Table 1. The result of eel fish’s albumin level analysis by heating (hotplate)

| Types of samples | Albumin level (g/L) | Average ±SD | Average g/100g |
|------------------|---------------------|--------------|---------------|
|                  | I | II | III       |               |              |
| A.m (0,5kg)      | 0.8| 0.8| 0.7       | 0.77±0.06     | 0.11         |
| A.m (0.7kg)      | 0.9| 0.7| 0.7       | 0.77±0.11     | 0.11         |
| A.b (0.5kg)      | 0.4| 0.4| 0.4       | 0.40±0.03     | 0.06         |
| A.b (0.7kg)      | 0.9| 0.9| 0.7       | 0.83±0.12     | 0.12         |

Note: 
A.m : Anguilla marmorata (Q.) Gaimard
A.b : Anguilla bicolor
Table 2. The result of eel fish’s albumin level analysis by heating (water bath)

| Types of samples | Albumin level (g/L) | Average ±SD | Average g/100g |
|------------------|---------------------|-------------|---------------|
|                  | I       | II  | III |                     | |
| A.m (0.5kg)      | 1,1    | 1,0 | 0,9 | 1,00±0,10            | 0,15 |
| A.m (0.7kg)      | 1,3    | 1,3 | 1,2 | 1,27±0,06            | 0,19 |
| A.b (0.5kg)      | 0,9    | 0,8 | 0,8 | 0,83±0,06            | 0,12 |
| A.b (0.7kg)      | 1,1    | 1,1 | 1,0 | 1,07±0,06            | 0,16 |

Note:
A.m : Anguilla marmorata (Q.) Gaimard
A.b : Anguilla bicolor

Both figures show differences in albumin levels on different extraction methods in statistical data. The water bath extraction acquires higher average albumin value of ±SD 1.042±0.1730g/L or 0.156g/100g compared to hotplate extraction which acquires average value of ±SD 0.692±0.1929g/L or 0.104g/100g. Then, according to the comparative test of albumin level using the t-test, there is a significant difference in albumin level between the hotplate and water bath extraction method.

The water bath is a method of extraction that uses steam heating in an isolated vessel. It gives the advantage of more albumin compared to its counterparts [18]. Moreover, the steam heating process should be controlled carefully because the too hot temperature may result in protein denaturation (a reduced chemical substance in the extract). Further, [19] states that denaturation is a process of spoiling the original characteristics of a substance by various factors in which the changes may occur in several ways such as chemical, function, and physical. Furthermore, albumin is recognized to be also to mix with various solvent, as stated by [20] that albumin is a globular protein that can dissolve with water, acid, or salt solvent but water solvent is considered better to extract albumin compared to acid solvent.

Based on the type of fish, eel fish type which possesses the highest albumin level is Anguilla marmorata (Q.) Gaimard with an average value ± SD 0.950 ± 0.2276g / L or 0.143g / 100g compared to eel fish type Anguilla bicolor with an average value ± SD 0.783 ± 0.2588g / L or 0.117g / 100g. Comparative test results on the average value using the t-test, indicating a significant difference between the levels of albumin eel Anguilla marmorata (Q.) Gaimard with Anguilla bicolor.

Then, based on the weight of 0.5 kg, eel fish with the highest albumin level is Anguilla marmorata (Q.) Gaimard with the average value ± SD 0.883 ± 0.1472g / L or 0.132g / 100g compared to Anguilla bicolor the average value ± 0.617 ± SD 0.2401g / L or 0.093g / 100g. Comparative test results on average by using the t-test, indicating a significant difference between the levels of albumin Anguilla marmorata (Q.) Gaimard with Anguilla bicolor. Moreover, on the weight of 0.7 kg, the highest albumin level is still on Anguilla marmorata (Q.) Gaimard with the average value ± SD 1.017 ± 0.2858g / L or 0.153g / 100g compared to Anguilla bicolor with the average value ± SD 0.950 ± 0.1517g / L or 0.143g / 100g. Comparative test results on average by using the t-test, indicating no significant difference between the albumin level of Anguilla marmorata (Q.) Gaimard with Anguilla bicolor. Furthermore, differences in levels of albumin Anguilla marmorata (Q.) Gaimard by weight. The highest albumin levels on the weight of 0.7 kg with the average value ± SD 1.017 ± 0.2858g / L or 0.153g / 100g compared to 0.5 kg with the average value ± SD 0.883 ± 0.1472g / L or 0.132g / 100g. Comparative test results on the average value using the t-test, indicating no significant differences between the levels of albumin eel Anguilla marmorata (Q.) Gaimard is based on the weight of 0.5 kg and 0.7 kg weight. In the other hands, differences in albumin level of Anguilla bicolor type based on the weight of 0.7 kg weight the average value ± SD 0.950 ± 0.1517g / L or 0.143g / 100g compared to 0.5kg with the average value SD ± 0.617 ± 0.2401g / L or 0.093g / 100g. Comparative test results on the
average value using the t-test, indicating a significant difference of albumin level between *Anguilla bicolor* type of weight 0.5 kg and 0.7 kg.

The findings of the research suggest that *Anguilla marmorata* (Q.) Gaimard contains the highest level of albumin which can be used as the body’s source of nutrition. Generally, freshwater fishes extract contains arginine and glutamic in a considerable amount and useful as an intracellular antioxidant [20]. Studies concerning the albumin level of catfish are at the top of the trend knowing that albumin of catfish proven clinical trials beneficial for health, especially for patients of hypo albumin (Hamal, 2009), HIV AIDS [21], and burns [22]. Also, albumin contains glutamine which is believed to be an energy source for cells that can improve the function of GALT (*gastrointestinal associated lymphoid tissue*) [23; 24] and acts as a negative regulator essential for the stimulation of inflammation, inhibiting phosphorylation [25]. Arginine is a precursor for a polyamine synthesis of collagen in wound healing and will also stimulate the release of the anabolic hormone. The role of arginine in the body’s immune system is mainly mediated by the formation of nitric oxide. Arginine supplementation 2% of the total calories in animal burns followed by an increased survival rate significantly [26].

![Figure 1. The average value of albumin level of eel fish (g/L)](image1)

![Figure 2. The average value of albumin level of eel fish (g/100g)](image2)
4. Conclusion
Concluding the preceding explanations, the researcher suggests that steaming extraction produces the highest albumin level compared to its counterpart. The highest albumin levels based on the type found in *Anguilla marmorata* (Q.) Gaimard. The highest albumin levels based on the weight is eel fish weight of 0.7 kg.

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