Research Article

CHARACTERIZATION OF THE PHYSICOCHEMICAL PROPERTIES OF ALBUMIN EXTRACT OF SNAKEHEAD FISH (*OPHIOCEPHALUS STRIATUS*) FROM RIVERS IN INDONESIA AS A SOURCE OF ALTERNATIVE ALBUMIN NUTRIENTS IN RAW MATERIALS FOR PHARMACEUTICAL PRODUCTS

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Article Received on: 26/02/19 Approved for publication: 19/03/19

DOI: 10.7897/2230-8407.100384

ABSTRACT

The purpose of this study was to determine albumin levels, and characterization of physicochemical properties of fractions containing snakehead fish albumin (*Ophiocephalus striatus*). This characterization can help standardize quality in the manufacture of pharmaceutical products containing albumin derived from snakehead fish. Fresh of snakehead fish are extracted using the freeze dryer method. The crude extract obtained was fractionated using gel filtration column chromatography using the sephadex G-100. The fraction containing albumin was carried out to determine the level and characterization of physicochemical properties, including: solubility, pH of the isoelectric point, optimum temperature. The crude extract contains a protein content of 68.72%, and albumin levels in the fraction of 13.72%. Albumin was detected in the fifth, sixth, and seventh fractions. Albumin fraction also has high solubility with the addition of distilled water or salt solution. pH isoelectric point of snakehead fish albumin fraction at pH 4.5. The optimum temperature of snakehead fish albumin fraction is 50°C, and albumin denaturation temperature at 50°C. Physicochemical characteristics of snakehead fish extract powder meet the requirements as an alternative source of raw materials for pharmaceutical products.

Keywords: albumin, snakehead fish (*Ophiocephalus striatus*), standardization of quality, physicochemical properties, solubility, isoelectric point, optimum temperature.

INTRODUCTION

Prevalence and the effects of malnutrition are a serious problem in all hospitals. Human albumin (HA) is the most expensive non-blood plasma replacement substance used to treat hypoalbuminemia. This condition of malnutrition has become an important issue in pregnant, infant, pediatric and geatric women. Because of its very limited availability and high costs, the use of HA is limited to indications that are very effective¹.

The albumin concentration in healthy human plasma ranges from 33 and 52 g liters - 1. Albumin is synthesized exclusively in the liver. The normal level of albumin synthesis is ,20.2 g kg - 1 body weight per day². Albumin is the highest plasma protein in the amount of about 60% and has various functions that are very important for health, namely the formation of new cell tissue, accelerating recovery of damaged body cell tissue and maintaining fluid balance in blood vessels with fluid in the interstitial cavity within the normal limits, blood albumin levels 3.5-5 g dl-1. Lack of albumin in serum can affect binding and transport of endogenous and exogenous compounds, including drugs, because as expected the distribution of drugs throughout the body is bound through albumin fraction^{3,4,5}.

Snakehead fish is a fish that has a high level of albumin. The results study was tested in a nutrition installation and surgical section of the General Hospital of Saiful Anwar Malang. The trial was carried out in postoperative patients with low albumin levels (1.8 g dl-1) ⁶.

Nowadays, pharmaceutical products from head fish are developed by researchers in the form of cream preparations⁷, capsule preparations⁸ and suspension preparations⁹. Research on the characterization of physicochemical properties of snakehead fish extract raw material is an effort to determine quality control standards for pharmaceutical industry producers in producing pharmaceutical products containing albumin extract from snakehead fish.

MATERIALS AND METHOD

Materials

Snakehead fish extract powder (*Ophiocephalus striatus*) from PT. Royal Medicalink Pharmalab Indonesia, sephadeks G-100, brocresol green, bromophenol blue, β-mercaptoetanol, polyacrylamide gel, coomasie blue, folin-ciocalteu were bought from PT. Bratachem. All other ingredients used were of analytical grade. Column gel filtration, heratherm incubator (Thermoscientific), eppendorf heat block, vokam 2541 power supply and electrophoresis control unit, UV Spectrophotometry (Infinite M200 Pro TECAN).

The organoleptic test

Organoleptic testing was carried out by observing the taste, smell, and appearance of snakehead fish extract powder to 25 panelists.

Determination of Protein Levels

Determination of protein content in albumin extract powder was carried out by the lowry method using UV spectrophotometry at

the wavelength of 630 nm¹⁰. The reagent used is lowry reagent which consists of:

Reagent A : Na₂CO₃ 2% and NaOH 0.4%.

Reagent B : 0.5% CuSO₄ and 1% Na-K Tartrate,

Purification and Determination of Albumin Levels

Separation of snakehead fish albumin extract powder using gel chromatography method using Sephadex G-100 matrix. The solvent system used in this filtration gel is Chloride Acid, monobasic potassium phosphate (KH₂PO₄), potassium phosphate dibasic (K₂HPO₄). The filtrate obtained was then identified and measured by using a UV spectrophotometer at 630 nm¹¹.

Isoelectric Point Testing

The filtrate from the gel filtration process containing albumin was examined for its isoelectric point using electrophoresis with an electric current system (2.5 mA), voltage (110 volts), time (20 hours)¹².

Solubility Testing

Solubility testing was carried out on fractions containing albumin. The fraction was dissolved into several solvents including: Aquades, NaOH, NaCl, Ethanol and Chloroform. Observations are made at the speed of the dissolving process and the condition of the solution formed¹³.

Optimal Temperature Testing

The optimal temperature of albumin has been widely investigated using human serum albumin (HSA), and bovine serum albumin (BSA). Albumin fraction from gel filtration column chromatography was dissolved using BPS solvents, then placed on eppendorf heat block with temperature variations 25°C, 37°C, 40°C, 45°C, 50°C, 55°C, 60°C and 65°C. After 30 minutes, then albumin fraction was measured by a UV spectrophotometer at absorbance 630 nm wavelength^{14,15}.

Stability Test

Tests were carried out using standard albumin and albumin fractions with the same concentration, and incubated at 50°C. Every 30 minutes, 50µl is taken, then the bromocresol green indicator is added according to the optimum pH of each. Then the absorbance value is tested, and done until the absorbance value is close to zero. The half-life is obtained based on residual absorbance (50% of initial activity) with respect to the incubation time.

RESULTS AND DISCUSSIONS

Organoleptic Testing

The organoleptic test of snakehead fish extract powder from PT Royal Medicalink Pharmed (Indonesia) produced data that 100% of panelists like the color and smell. A total of 91% of panelists like taste, and only 50% of panelists like the texture. This is because the texture of the powder of corm fish extract is not too smooth, and tends to be moist. Improvement of the production process, especially optimization of drying temperatures and refining processes using mesh size optimization will greatly affect the results preferred by panelists.

Protein Level Testing

The results of testing the levels of albumin extract powder protein using BSA as standard, absorbance data were obtained as follows:

Table 1: The BSA standard concentration for the Standard curve

Concentration (ppm)	Absorbance
0	0
100	0.0103
200	0.0149
400	0.0344
800	0.0682
1600	0.1389

Figure 1: Standard Curve BSA with The Lowry Method

The results of physicochemical characterization tests showed that the protein content of snakehead fish extract powder was 19.83%. Decreased protein content of 6.12% when compared with protein content in the condition of fresh snakehead fish occurred during the process of the extraction process by spray drying. This snakehead fish protein value is higher than mackerel fish extract, which is 15.57%, using the same extraction method¹⁶.

Albumin Purification and Determination of Albumin Fraction Levels

The purification process from corm extract powder using the gel filtration column chromatography method with the Sephadex G-100 matrix. This method is a separation based on particle size. The size of the Sephadex G-100 matrix pores is 4000-100,000 Dalton. Proteins whose molecular size is greater than 100,000 Da will pass through the gaps between the matrices, and albumin with a molecular weight range of 66,000 will be retained in the pores of the matrix. In this study albumin came out in the mid fractions (5-7 fractions). From this stage 9mL albumin fraction was obtained with the acquisition of about 1,178 ppm and 13.72 g/dl concentrations.

Figure 2: Standard Curve Using Albumin Kit (Bromocresol Green)

Isoelectric Point Testing

The test results of the isoelectric point of albumin purification are at pH 4.5. In figure 3, it can be seen at pH 4.5 that the protein is right at the isoelectric point.

Figure 3: Isoelectric Point Test Results

Native albumin has pI isoelectric of 4.7, while cork fish albumin has pI of 4.5. The test results of the isoelectric point of albumin purification are at pH 4.5. In figure 3, it can be seen at pH starting from 4.4 that the protein begins to migrate near the isoelectric point, and right at pH 4.5 the protein is right at the isoelectric point. Proteins begin to migrate to the poles more positively at pH 4.6. This is in accordance with previous research¹⁷.

Solubility Testing

Solubility testing showed that albumin extract powder from snakehead fish soluble with distilled water, and NaCl. The solubility of albumin extract with NaOH forms a bubble but disappears after a few minutes. Albumin is not soluble in organic solvents, for example chloroform solvents. Albumin will denaturate when dissolved in ethanol solvent. The results of the albumin solubility test can be seen in figure 4.

Sample	Solvent				
Albumin Fraction 50µl + PBS	Aquades (dissolved)	NaOH (dissolved)	NaCl (dissolved)	Ethanol (not dissolved)	Chloroform (not dissolved)

Figure 4: Albumin Fraction Solubility Test Results

Albumin is a protein that is strongly influenced by physical and chemical factors from its environment. Albumin solubility is strongly influenced. Changes that occur in the structure of the protein are denatured. Things that can cause protein denaturation are extreme pH conditions, high salt concentrations, and organic solvents. Albumin dissolves completely with distilled water, NaCl. The solubility of albumin extract with NaOH forms a

bubble but disappears after a few minutes. Albumin is not soluble in organic solvents, for example chloroform solvents. Albumin will denaturate when dissolved in ethanol solvent¹⁸.

Optimal Temperature Testing

Albumin fraction from gel filtration column chromatography was dissolved using BPS solvents, then placed on eppendorf heat

block with temperature variations 25°C, 37°C, 40°C, 45°C, 50°C, 55°C, 60°C and 65°C. After 30 minutes, then albumin fraction was measured by a UV spectrophotometer at absorbance 630 nm wavelength. Based on these tests, the optimum temperature of albumin increased from 37°C to 45°C. Albumin is stable at the optimum temperature of the human body and reaches the optimum temperature at 45 °C.

Figure 5: Optimum Temperature Curve of Snakehead Fish Albumin Fraction.

The optimum temperature test aims to find out at what temperature the albumin fraction still has good conditions. Albumin easily undergoes coagulation, at high temperatures. When heated at temperatures greater than 50°C, albumin quickly forms hydrophobic aggregates which cannot be returned to its monomers¹⁹.

CONCLUSION

The results of physicochemical characterization tests of cork fish extract powder obtained excellent organoleptic test results for color, odor and taste parameters, while texture parameters still had to be improved in terms of particle size and powder moisture. Protein content of cork extract powder was 19.83%, and albumin content was 13.72g / dl. The cork albumin has a pH of isoelectric point of 4.5, which has very good solubility in the solvent Aquades, NaCl, NaOH. Whereas in solvents Ethanol and chloroform albumin do not dissolve. Albumin has an optimum temperature at 45 °C.

ACKNOWLEDGEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper. The authors gratefully acknowledge to thank Mr. Ade Imanudin from PT Royal Medicalink Pharmed Indonesia for this research collaboration. The author also thanked Heina Arokiadass for her contribution to this research.

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Cite this article as:

Danni Ramdhani et al. Characterization of the physicochemical properties of albumin extract of snakehead fish (*Ophiocephalus striatus*) from rivers in Indonesia as a source of alternative albumin nutrients in raw materials for pharmaceutical products. *Int. Res. J. Pharm.* 2019;10(3):84-87 <http://dx.doi.org/10.7897/2230-8407.100384>

Source of support: Nil, Conflict of interest: None Declared

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