



Albumin, as a Therapeutic Protein: Potential Source, Application, Isolation and Purification

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ABSTRACT

Albumin is one of the plasma proteins found in the human body, which is about 55-60%, and the total normal serum protein level is 3.8-5.0 g/dL. Albumin consists of a single chain of a polypeptide with a molecular weight of 66.5kDa and consists of 585 amino acids. In the albumin molecule, 17 disulfide bonds connect amino acids containing sulfur. The molecular albumin is elliptical so that with such molecular forms, it will not increase the plasma viscosity and dissolve perfectly. Albumin is protein and its action as a transport agent and maintains colloid osmotic pressure. The need for albumin in the world reaches 500 tons annually, which is used hypoalbuminemia therapy. Hypoalbuminemia therapy which is often used is HSA (Human Serum Albumin) therapy. The use of HSA has disadvantages such as causing infections, expensive processing costs, and the cost of products. Thus other sources of albumin are needed. One that can be the potential source of albumin is Indonesian catfish (*Clarias-gariepinus*). Our research studies have obtained the albumin from Indonesian catfish (*C. gariepinus*) and Patin fish (*Pangasianodon hypophthalmus*) with high yield and purity. The Indonesian catfish is the potential albumin source after the isolation and purification process was obtained that the molecular weight, the purity, and the total albumin content of purified albumin were 66.7 kDa, 95.38%, and 118.5 mg /g of wet weight, respectively.



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INTRODUCTION

Albumin is one of the plasma proteins found in the human body, consists of a single chain of the polypeptide with a molecular weight of 66.5kDa and consists of 585 amino acids (Nicholson *et al.*,

2000). In human, the protein binds a wide variety of endogenous ligands, including non-esterified fatty acids, bilirubin, hemin and thyroxine (Peters, 1996). The molecular albumin is elliptical so that with such molecular forms, it will not increase the plasma viscosity and dissolve perfectly. Serum albumin levels are determined by the function of synthesis rate, degradation rate, and distribution between the intravascular and extravascular compartment. Reserve total albumin of 3.5-5.0 g/kg body weight or 250-300 g in healthy adults with a weight of 70 kg; of this number, 42% are in the plasma compartment and the rest in the extra-vascular compartment. Many attempts have been made to isolate pure and high-quality albumin to be utilized for therapeutic and research. Until now, various researchers and teams have tried to innovate in discovering new albumin production methods. Tradi-

tional techniques such as fractionation have been developed and diverse chromatography techniques are currently used to achieve albumin with high yield and purity (Raoufinia *et al.*, 2016).

Human Albumin (HA) synthesis process is stimulated by hormones, such as insulin, cortisol, and growth hormone, while it is inhibited by pro-inflammatory substances, including interleukin-6 and tumor- α necrosis factors (Evans, 2002; Nicholson *et al.*, 2000). Albumin has a strong negative charge but binds weakly and reversibly to both cations and anions. It, therefore, functions as a circulating depot and transports molecules for a large number of metabolites, including fatty acids, ions, thyroxine, bilirubin, and amino acids (Evans, 2002). Albumin in the reduced state contains a single exposed thiol group (King, 1961), which is the principal extracellular antioxidant and chiefly responsible for maintaining the redox state of plasma. The influence of hormonal changes, for example, raised concentrations of insulin, thyroxine, and cortisol, can influence albumin synthesis (Kimball *et al.*, 1995). Surprisingly, growth hormone has no such effect. HA molecules also contribute to the stabilization of the endothelial layer and maintain normal capillary permeability conditions, probably by reducing oxidative damage as well as modulate inflammation (Kitano *et al.*, 1996; Chen *et al.*, 2009).

Human Serum Albumin (HSA) binds and carries a wide variety of hydrophobic molecules, besides endogenous molecules such as cholesterol, fatty acids, bilirubin, and thyroxine, or exogenous substances such as drugs and toxins, as well as transition metal ions, and nitrogen oxide gases. With consequent implications for their solubilization, transportation, metabolism, and detoxification (Fanali *et al.*, 2012; Garcia-Martinez *et al.*, 2013). HSA has been used as one of the therapies in the recovery of hypoalbuminemia conditions. However, the use of HSA is still a debate for scientists, especially in the health field. Because HSA has some disadvantages that provide infections for patients who are not suitable, doses and indications are unclear, high production costs resulting in the price of the product becomes expensive (Xu *et al.*, 2013; Jatiningsih *et al.*, 2015).

Hypoalbuminemia is a condition where blood albumin levels are less than 3.5 g/dL. In the condition of hypoalbuminemia, there will be disturbances to the processes of physiology in the body, especially in patients who are severely ill, to interfere with or inhibit the healing and recovery process. There is a connection between low albumin levels and increased risk of infectious complications, pro-

longed wound healing, prolonged hospitalization, high mortality rates in inpatients, both patients who do not operate or operating sufferers (Nicholson *et al.*, 2000).

Albumin function and Application

The main application and function of albumin, in particular as a plasma protein that acts as an effective buffer, transporting drugs and endogenous compounds, has numerous functions in health, exhibits significant antioxidant (Nicholson *et al.*, 2000; Oettl and Stauber, 2007). Albumin, as the major plasma protein, is expected to be a target of modification during oxidative stress (Oettl and Stauber, 2007). The Structural basis of the drug-binding specificity of Human Serum Albumin have been published by Ghuman *et al.* (2005). The protein binds a wide variety of endogenous ligands, including nonesterified fatty acids, bilirubin, hemin and thyroxine (Peters, 1996). Albumin is widely used for volume replacement and treating hypoalbuminemia (Uhing, 2004; Boldt, 2010).

Serum albumin is utilized under various clinical conditions. Restoration of blood volume, emergency treatment of shock, acute management of burns, and other situations associated with hypovolemia are some of the clinical applications of albumin (Raoufinia *et al.*, 2016).

Endogenous plasma albumin is a viable target by which drug developers have sought to manipulate the solubility, distribution, pharmacokinetics, and pharmacodynamics of exogenously administered drugs, the use of plasma-derived albumin to modify the same *ex vivo* has met with increasing safety concerns. To meet these needs, many systems have been assessed for the manufacture of recombinant albumin for therapeutic applications (Fanali *et al.*, 2012).

Albumin can bind to a variety of substances such as drugs, long-chain fatty acids, bilirubin, bile acids, endotoxin, hormones, and eicosanoids, modulating their biologic activity, distribution, and clearance (Levitt and Levitt, 2016). Treatment with albumin has been widely used in liver cirrhosis due to its oncotic properties, to expand plasma volume and to increase effective circulatory volume, and hence to abrogate the cardio-circulatory changes associated with portal hypertension (de Mattos, 2011).

Human albumin has the highest demand among other biopharmaceutical solutions. Currently, the annual request for albumin is guessed by approximately 500 metric tons in the world (Chen *et al.*, 2013). Human Serum Albumin (HSA) is one of the expensive drugs associated with limitations in

the market and difficulty in the production process. The procurement of albumin, especially for surgical cases, reaches 91%, where albumin is used in healing therapy in post-surgical patients and also in patients with internal medicine. It is estimated that as much as \$40,000 has been spent to obtain healing therapy with albumin (Vargas *et al.*, 1997; Alexander, 1982).

Cork fish extract containing Fish Serum Albumin (FSA) has become a necessary product in some hospitals as a companion to treatment to accelerate wound healing. Mustafa *et al.* (2012) reported the FSA extract from fish is also needed to meet the needs of serum albumin on the handling of hypoalbuminemia disease in an increasingly elevated hospital, as an alternative to synthetic HSA, which has a relatively expensive price (Hasnain *et al.*, 2004; Jais, 2007).

Human Serum Albumin recombinant

Recombinant Albumin (rHSA) Expression in *Escherichia coli* BL21(DE3).

Maksum *et al.* (2019) have done expression of recombinant Human Serum Albumin (rHSA) in *E. coli* BL21(DE3) using TorA signal peptide for Human Albumin secretion.

Lysate from six *E. coli* BL21(DE3) [pD881-torA-HSA] transformant colonies showed that HSA was expressed in the cytoplasm. It was characterized by the presence of ± 67.0 kDa as shown in the electropherogram of SDS-PAGE (Maksum *et al.*, 2019).

Latta *et al.* (1987) expressed recombinant human albumin (rHA) in *E. coli* and produce rHA that has similarity to HSA native after renaturation and disconnection of Met-HA by trypsin. Expression of the rHA in the *E. coli* host requires a renaturation process that takes time, expensive costs, and often proteins produced in the form of inclusion bodies and has no post-translation modifications.

Recombinant Albumin (rHSA) Expression in *Pichia pastoris* and *Saccharomyces cerevisiae*

P. pastoris shows attractive potential for HSA expression under several advantages, including the simplicity of its molecular genetic manipulation, its genetic stability, its strong inducible alcohol oxidase 1 (*AOX1*) promoter, the high cell density it displays in an inexpensive basal salt medium, its potential of posttranslational modification, its high secretory capacity and its low endogenous protein secretion. Recently, Zhu *et al.* (2018), reported the high-level production of rHSA in *P. pastoris* GS115 hosts reached 1.6 g/L in a shake flask and 8.86 g/L in a fermenter. Prevatt and Sreekrishna (1994) have done the expression and optimization of rHA pro-

duction in *P. pastoris* strain GS115 and obtained a fairly high result of 2.90 g / L rHA at the condition of pH 5.97. The use of the *P. pastoris* seems to the best result for rHA expressions that have 17 disulfide bonds, where the expression requires post-translation modification with a high level of expression (Latta *et al.*, 1987). Meanwhile, the expression of rHA in the host of *S. cerevisiae* produces a low level of expression, possibly due to the large molecular weight of the rHA, so that part of the rHA is held in the periplasmic cavity and hyper-glycosylation often occurs. Okabayashi *et al.* (1991) expresses rHA in the host of *S. cerevisiae* and produces rHA of 0.085 g/L.

ALBUMIN IN FISH

Fish are foods that contain a good quality animal protein because of the complete essential amino acid content. Fish can be extracted to obtain plasma proteins (sarcoplasmic) containing albumin and other nutrients that can potentially improve the condition of patients with hypoalbuminemia (Mustafa *et al.*, 2012). The function of protein Fish Albumin (FA) is as one of the transport agents of metabolites in the body (fatty acids, hormones, bilirubin), regulating the system of osmoregulation in the body and also the osmotic pressure system of colloidal blood, as well as a filter of fluid in body tissues (De Smet *et al.*, 1998; Rudneva and Kovyrshina, 2011). This Protein has a characteristic with a negative charge with pI = 4.7 and a relatively low molecular weight of 66.4 kDa (De Smet *et al.*, 1998; Baker, 2002). The amount of protein such as FA in fish meat varies depending on the type of fish, size, physiological status, feeding level, environment, and feed quality, as well as the energy content that can be digestible on the feed. Besides, the FA levels in fish are also influenced by genetic factors (Hasnain *et al.*, 2004; Rudneva and Kovyrshina, 2011). It plays a role in osmoregulation (Zhang *et al.*, 2005).

Albumin was found in the plasma of the bonnet-head shark *Sphyrna tiburo* at a concentration from 0.5% or around 13.5% of the total plasma protein that amounted from 2.2 to 43% (Harms *et al.*, 2002). Cork fish (*Channa striata*) is a freshwater fish that is known to have a high protein content, especially albumin. Mustafa *et al.* (2012) reported that levels of albumin and zink (Zn) in cork fish protein extract have important effects for health. The high content of albumin in cork fish causes this fish to have been used to overcome hypoalbuminemia (Mustafa *et al.*, 2012).

Today, albumin-based nutraceutical products from the Snake Head Fish (SHF), *Channa striatus* have

been developed in Indonesia and Malaysia due to their properties that improve the health status of hospitalized patients suffering from hypoalbuminemia and post-surgical tissue damage. Therefore, the demands for *C. striatus* in Indonesia and the Asian market have been increasing every day (Khasani and Astuti, 2019). SHF is a reputed medicinal freshwater fish in the South Asian region and used to treat wounds, alleviate pain and boost energy. It is endowed with remarkable anti-inflammatory, anti-nociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties. Its nutraceutical value is outstanding and contributes, at least in part, with its bioactive compounds, to clinical trials, therapeutics, and nutritional supplements (Siswanto et al., 2016; Rahman et al., 2018). Therefore, SHF has a high potential to be used as a source of nutrients for the treatment of serious diseases, as well as for the improvement of the general body tonus of humans (Courtenay and Williams, 2004). Related to the medical function, albumin is a major functional protein of the SHF.

FSA (Fish Serum Albumin) has become a commercial nutraceutical product because it has useful bioactivity for pharmacological needs. FSA-based nutraceutical products that have been produced in the market generally come from cork fish (*Chan-nastriata*). Traditionally, Cork has been known as wound healing, pain relievers, ACE (Angiotensin Converting Enzyme) inhibitors, anti-depression, and degenerative nerve disease (Mohd and Manan, 2012).

Albumin, following its divergence from the ancestral gene, has an evolutionary history of 400 million years (Gitlin et al., 1973) and shares several characteristics with other proteins of common origin and evolution. Due to functional overlaps with sister proteins of the multigene family, albumin cannot be unambiguously identified by any single criterion. As the above results demonstrate, some criteria have to be applied to elucidate the structural and functional properties of Catfish Serum Albumin (CfSA). The similarity in size and shape of CfSA and HSA is evident from the value of Stoke's radius (3.34 for CfSA), which is in good agreement with the reported value of 3.56 for BSA and HSA (Tayyab et al., 1991; Khan et al., 2000). Further, the Mr value of 70 kD obtained by SDS-PAGE is similar to that obtained for HSA and also for salmonid serum albumins (Robey et al., 1983; Maillou and Nimmo, 1993).

Susilowati et al. (2015) found that the content of albumin in the Catfish is 103.37 ± 1.53 mg/g, where the amount of this content is one of several fish that has a high albumin content (Susilowati et al., 2015).

Meanwhile, in the year 2016, Widyastuti stated that the content of albumin in the catfish was 13.67 mg/mL. Where the catfish occupies the second position as a fish containing the most albumin among several types of fish (Widyastuti, 2016). Albumin in the catfish has a measure aligned with the HSA of ± 67 kDa. Here size of catfish albumin aligned with the HSA shown on Hasnain et al. (2004).

Isolation, Purification and Characterization of Albumin from Indonesian Catfish (*C. gariepinus*)

Cold acetone and Ammonium sulfate precipitation combined with liquid chromatography

Characterization of protein in the acetone powder results using SDS-PAGE showed that albumin with a molecular weight of 66.7 kDa was successfully isolated. However, in the isolate of acetone precipitation results, there are still many contaminant proteins that was indicated by the amount of spot that appears in the SDS-PAGE result, so that further purification stage is needed (Figure 1). The 1st lane is the protein standard (marker), the 2nd lane is the crude extract, and the 3rd lane is the cold acetone precipitation result.

Fish albumin from Indonesian Catfish (*C. gariepinus*) could be isolated using the modified method. The catfish meat (150.0 g of wet meat) was sliced and homogenized in a 150 mL Tris-HCl buffer of 20 mM pH 7.6 and sodium chloride 0.9% and incubated for 24 hours at a \pm of 4°C. Later on, it was precipitated by the addition of cold acetone and followed by ammonium sulfate precipitation. Purification was done by gel filtration chromatography and ion-exchange chromatography. The purity of the isolated protein was monitored with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), and the determination of albumin content from isolates using the bromocresol green method. (Keaney et al., 1993; Kim et al., 2007; Sleep, 2015)

Ammonium Sulfate Precipitation

An aliquot of protein as the results of cold acetone precipitation was added with ammonium sulfate until the concentration reaches precisely saturated or 100%. This stage is carried out based on the results of Odunuga and Shazhko (2013), that albumin settles on the addition of ammonium sulfate with a concentration of 40-100%. At 100% ammonium sulfate saturation, all of the albumin from the sample will be precipitated. Then the solution was incubated overnight at 4°C to maximize the salting-out process between ammonium sulfate salts against albumin. The incubated solution was then concentrated at a speed of 1000 xg at 4°C for 15

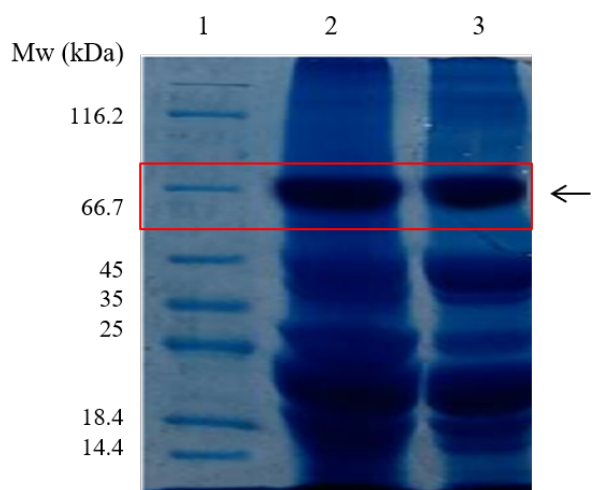


Figure 1: SDS-PAGE of acetone precipitation result of catfish protein

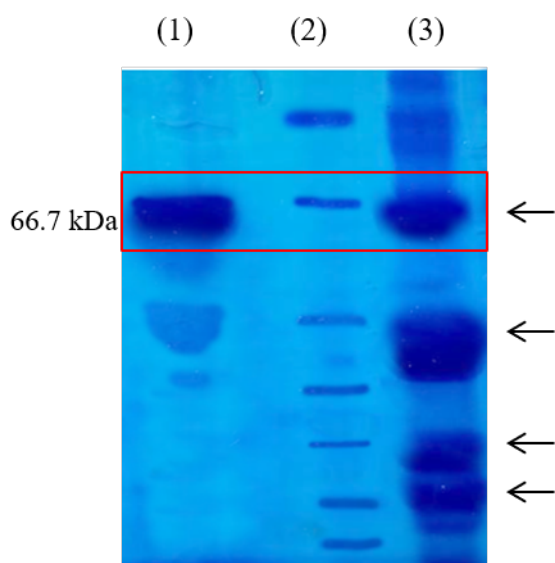


Figure 2: SDS-PAGE of ammonium sulfate precipitation result with 100% saturation

minutes. The precipitate was then dissolved with a 250 mL buffer containing Tris-HCl pH 9.20 mM and sodium chloride 100 mM. (Kim *et al.*, 1999)

Figure 2, shows that the result of ammonium sulfate precipitation with 100% saturation managed to isolate the albumin marked on the tape showing proteins with a molecular weight of ± 66.7 kDa. The 1st lane is the standard of Bovine Serum Albumin (BSA), The 2nd lane is the Marker (protein standards), the 3rd lane is the precipitation result of 100% saturated ammonium sulfate

However, there are still many proteins as impurities. The dominant proteins are proteins with a molecular weight of $\pm 45, 39$, and 25 kDa, which are precipitated by the addition of ammonium sulfate. This is also the case in research conducted by Odunuga

and Shazhko (2013) that some of the proteins that are deposited in the addition of ammonium sulphate with a certain concentration. The presence of impurities proteins can be eliminated by further purification using chromatographic methods.

Purification of albumin isolate using Sephadex G-50 gel filtration chromatography

Albumin isolates were purified by gel filtration chromatography using Sephadex G-50 matrix with the aim of desalting (Nadeem *et al.*, 2010). Isolates are resulting from the precipitation of ammonium sulfate, containing a high concentration of ammonium sulfate salts that must be removed first to facilitate the next purification process. This is following the principle of separation of gel filtration chromatography that separates proteins based on differences in molecular size or weight (Hong *et al.*, 2012).

Purification using gel filtration chromatography is done by valuing 5 mL of ammonium sulfate precipitation isolate into a column containing Sephadex G-50 matrix with an eluent 0.02 M buffer Tris-HCl pH 9 and accommodated every 5 mL with a flow rate of 5 mL / 9 minutes. The purification results are controlled by absorbance measurement of each fraction at wavelengths of 280 nm and 260 nm to determine the fraction containing proteins, as well as conductivity measurements to determine the fraction containing salt. At this stage, proteins that have a molecular weight greater than matrix pores will escape and be delusional first, while salts that have a molecular weight smaller than 30 kDa will enter the matrix pores and be delusional later.

The results of purification with gel filtration chromatography shown in Figure 3, indicate the separation between proteins and salts characterized by the formation of absorbance peaks at wavelengths of 280 nm and 260 nm in the presence of three peaks of absorbance, indicating the fraction contains proteins where the absorbance value of each peak is 0.102; 3,000; and 0.616. For peak conductivity is at the 36th fraction with a conductivity value of 187.12 $\mu\text{S}/\text{cm}$, where the peak with a high conductivity value indicates the presence of salt contained in the fraction.

Albumin purification using Ion Exchange Chromatography on DEAE-Sepharose

Purification using ion exchangers needs to be done to separate contaminant proteins from albumin based on isoelectric point differences because each protein has a specific isoelectric point value. A total of 5 mL of sample is diluted in DEAE-Sepharose matrix column in the form of 0.02 M buffer Tris-HCl pH 9 with increased concentration of sodium

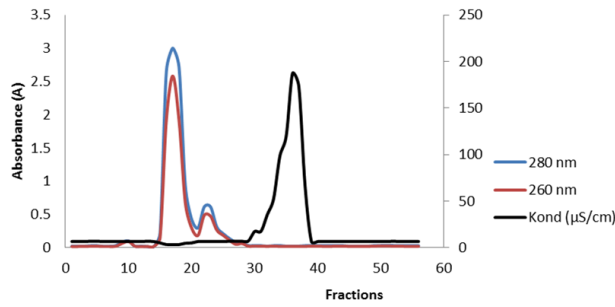


Figure 3: Protein profile of catfish using Sephadex G-50 gel filtration chromatography, diluted with 0.2 M buffer Tris-HCl pH 9 with a flow rate of 5mL/9 minutes

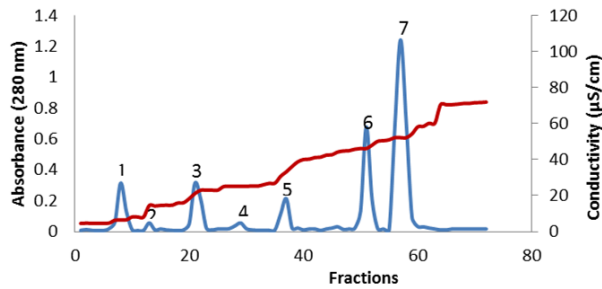


Figure 4: Absorbance graph of ion exchanger chromatography purification results of albumin fraction with DEAE Sepharose matrix and as the eluent is 0.02 M buffer Tris-HCl pH 9 containing 1M sodium chloride with salt concentration gradient method

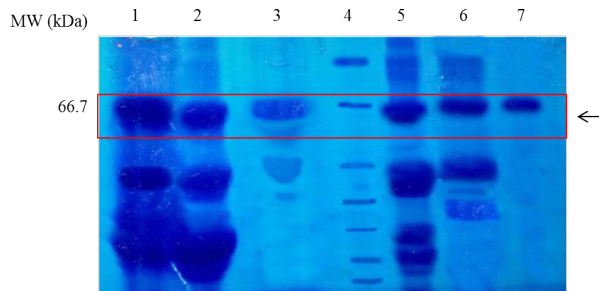


Figure 5: Electrophoregram of SDS-PAGE results from each stage of isolation and purification of Catfish albumin

chloride gradients up to 1 M, and each fraction accommodated every 5 mL. Each fraction is evaluated for its absorption at a wavelength of 280 nm. The results of purification with ion-exchange chromatography shown in Figure 4.

The electrophoresis result of SDS-PAGE in Figure 5 shows a purification summary of each stage of isolation and purification such as precipitation with acetone, precipitation with ammonium sulfate, gel filtration chromatography, and ion-exchange chromatography. The electrophoresis gel result shows that the protein impurities are decreasing from each

stage, which indicates that the purity of the albumin is increase. The SDS-PAGE results also obtained a single band at a size of ± 66.7 kDa, that indicating the albumin was successfully purified by using ion-exchange chromatography on DEAE Sepharose.

From Figure 5, the 1st lane is the crude extract, and the 2nd lane is the result of acetone precipitation, the 3rd lane is BSA, the 4th is the protein standard (Marker), the 5th lane is ammonium sulfate precipitation result, the 6th lane is the result of gel filtration, the 7th lane is the peak 7 of the ion-exchange chromatography

Analysis of total protein content with Lowry method

From all stages of research that has been done, obtained total albumin content in the ion exchange column of 11.85 g and obtained albumin levels that were successfully purified from 100 g of catfish meat of 118.5 mg / g from the wet weight. Albumin levels obtained from this study are higher than the levels of albumin catfish that have been studied by Susilowati et al. (2015), 103.37 mg/g of wet weight. This happens because of the different sources of catfish obtained.

The nutritional content of catfish depends on the size, physiological status, feeding level, environment, and feed quality, as well as the digestible energy content of the feed (Rosa et al., 2007; Hasnain et al., 2004). In addition, FSA levels in fish are also influenced by genetic factors (Hasnain et al., 2004; Rudneva and Kovyrshina, 2011). These factors led to differences in albumin levels obtained between researchers and research conducted by Susilowati et al. (2015).

Isolation, Purification and Characterization of Albumin from Indonesian Patin-fish (*P. hypopythalamus*)

Gel filtration chromatography result of Indonesian Patin-fish (*P. hypopythalamus*) protein shown on Figure 6, and the separation of albumin using ion-exchange chromatography from the first peak of gel filtration result shown in Figure 7.

Separation with ion-exchange chromatography requires the presence of charge-containing proteins in experimental conditions or when diluted. The protein will replace the low molecular weight ions of the anion exchange matrix and will be bound to the matrix. Changes in conditions during delusions (such as increased salt or counter-ion concentrations or decreased charge of protein ions) will cause the turnover of other ions, and the protein will be removed from the exchange matrix because it will bind to the counter-ion (Bollag and Stuart,

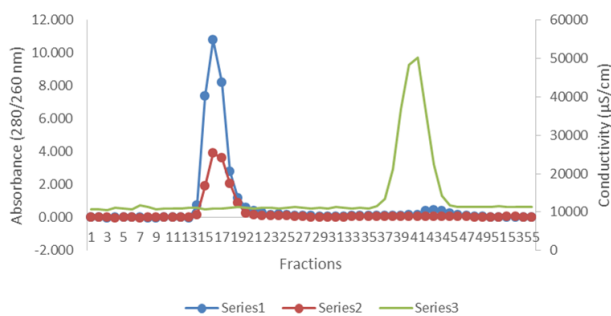


Figure 6: Protein profile of Patin-fish using Sephadex G-50 gel filtration chromatography, diluted with 0.2 M buffer Tris-HCl pH 9 with a flow rate of 5 mL/9 minutes. The blue and the red line indicates the absorbance of the fractions at 280 nm and 260 nm

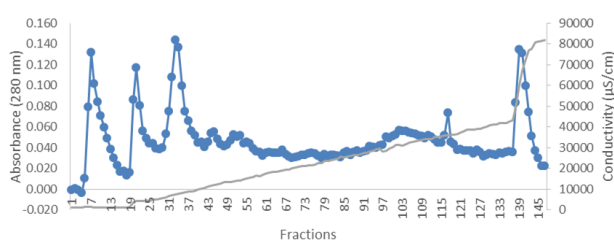


Figure 7: Absorbance graph of ion exchanger chromatography purification results of albumin fraction with DEAE Sepharose matrix and as the eluent is 0.02 M buffer Tris-HCl pH 9 containing 1M sodium chloride with salt concentration gradient method

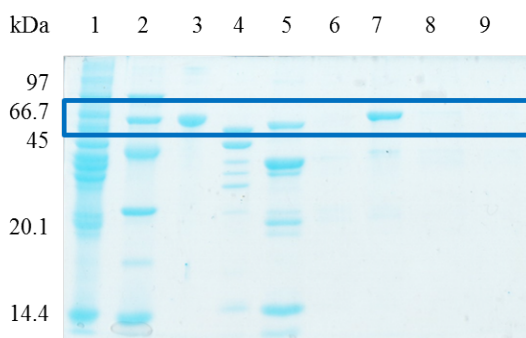


Figure 8: Electrophoregram of SDS-PAGE results from each stage of isolation and purification of Patin-fish albumin.

1998). The peak fraction of the ion-exchange chromatography were characterized by using SDS-PAGE electrophoresis (Figure 8).

In Figure 8, it appears that there is a ribbon parallel to the BSA band of protein markers at fractions 96-99 (lane 7). It is obtained a single band at a size of ± 66.7 kDa, that indicating the albumin from Patin-fish was also successfully purified by using ion-exchange chromatography on DEAE Sepharose. But, the content of albumin in Indonesian Patin-fish

was lower than albumin of Indonesian catfish.

From Figure 8, the 1st lane is the crude extract, the 2nd lane is the result of standard protein (marker), the 3rd lane is BSA, the 4th is the fractions of 10-13, the 5th lane is fractions of 52-56, the 6th lane is fractions of 83-86, the 7th lane is fractions of 96-99, the 8th lane is fractions of 107-110, and the 9th lane is fractions of 111-114

CONCLUSION

Albumin is a very important substance and is widely used for volume replacement and treating hypoalbuminemia, and also as an extremely versatile and effective platform in drug delivery. The expression of Recombinant Albumin (rHSA) in the *E. coli* BL21(DE3) and the *P. pastoris* showed attractive potential and good for HSA expression. The albumin of the snakehead fish (SHF) was used to treat wounds, alleviate pain and boost energy. It is endowed with remarkable anti-inflammatory, antinociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties. The corks-fish extract containing Fish Serum Albumin (FSA) has become a necessary product in some hospitals as a companion to treatment to accelerate wound healing. The Indonesian catfish is the potential albumin source after the isolation and purification process obtained that the molecular weight, the purity, and the total albumin content of purified albumin were 66.7 kDa, 95.38%, and 118.5 mg /g of wet weight, respectively.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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