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COMPARISON OF L-ASPARAGINASE ENZYME PURIFICATION METHODS FROM RECOMBINANT ESCHERICHIA COLI FOR LEUKEMIA THERAPY: A REVIEW

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ABSTRACT

L-asparaginase is an enzyme used for cancer therapy because it can hydrolyze L-asparaginase into aspartic acid and ammonia, which can cause cancer cells to die due to loss of nutrients. L-asparaginase in nature is found in algae, plants, and microbes. *Escherichia coli* is used as the first line in the treatment of leukemia. The production of recombinant protein in *Escherichia coli* has several advantages, including effectiveness and low cost as well as easy transformation and fermentation. The purpose of this scientific article review is to compare the L-asparaginase Enzyme Purification Method from Recombinant *Escherichia coli* for Leukemia Therapy. Purification of L-asparaginase from *Escherichia coli* showed the highest specific enzyme activity was 312.8 U/mg by DEAE Sepharose ion chromatography method.

KEYWORDS: L-asparaginase, *Escherichia coli*, Purification.

INTRODUCTION

Cancer is a malignancy caused by uncontrolled cell growth and abnormal spread to other organs.[1] One type of cancer that often occurs in the world is blood cancer (leukemia) which is characterized by the proliferation of white blood cells with the manifestation of abnormal cells in the peripheral blood (blast cells) in excess, resulting in the urgency of normal blood cells resulting in impaired function.[2] The prevalence of leukemia in the world was 2.4 % of new cases and 3.2 % of deaths in 2018.[3] Meanwhile, in 2019 there were 27,380 new cases of acute leukemia.[4]

Some anticancer drugs that can use as chemotherapy include alkylators, antimetabolites, antibiotics, hormones, microtubule protein inhibitors, topoisomerase inhibitors, and molecular target groups.[5] In addition to using chemical-based chemotherapy, another alternative that can be used is enzyme therapy. In the medical field, the L-asparaginase enzyme has been used for leukemia therapy.[6] L-asparaginase is an enzyme used for cancer therapy because it can hydrolyze L-asparaginase into aspartic acid and ammonia, so that it can cause cancer cells to die due to loss of nutrients.[6]

L-asparaginase in nature is found in algae, plants, and microbes.[7] Microbes are one of the better sources for the production of L-asparaginase because they are easy to culture, thus enabling large-scale production.[8] Some of the L-asparaginase-producing bacteria are *Escherichia coli*,[9] *Erwinia chrysanthemi*,[10] *Enterobacter aerogenes*,[11] *Pseudomnas aeruginosa*,[7] *Bacillus licheniformis*,[12] *Thermus thermophiles*,[13] *Staphylococcus aureus*,[14] besides bacteria there is a L-asparaginase-producing fungus, *Aspergillus terreus*,[15] *Penicillium brevicompactum*,[16] *Mucor hiemalis*.[17] From various sources explored L-asparaginase from *Escherichia coli* was approved as a drug for leukemia by the FDA in 1978,[18] and was used as the first line in the treatment of leukemia.[4] The production of recombinant protein in *Escherichia coli* has several advantages, including effectiveness and low cost as well as easy transformation and fermentation. In this review article, we will discuss the comparison of the L-asparaginase enzyme purification method from Recombinant *Escherichia coli* for Leukemia Therapy. Enzyme purification is important because it removes some contaminant compounds that can affect enzyme work, for example inhibiting enzyme activity (inhibitors) or having similar capabilities to the target enzyme but producing different or unwanted products.[19]

MATERIALS AND METHODS

This article review was conducted based on data obtained from primary and secondary libraries. Primary libraries are obtained from original articles published in scientific journals indexed by Scopus, Web of Science, DOAJ, EBSCO, and Google Scholar. Secondary libraries are obtained from electronic books and textbooks. Literature was obtained from scientific articles in Google Scholar, Science Direct and Pubmed using the keywords "L-asparaginase" "*Escherichia coli*" and research tested such as "purification method". The stages of reviewing scientific articles carried out include determining the topic of review of

scientific articles, determining the scope, determining the database, searching for literature, conducting a literature review, and writing summaries and discussions.

RESULTS AND DISCUSSION

In the literature review and discussion, the method of purification of the L-asparaginase enzyme from *Escherichia coli* is explained for the treatment of leukemia.

Table 1: Research results regarding the L-asparaginase enzyme purification method from *Escherichia coli* for the treatment of leukemia.

Bacteria	Step purification	Purification method	Specific enzyme activity (U/mg)	Literature
Escherichia coli	supernatant	Precipitation	8.2	[20]
	Ammonium sulfate (80 %)	Precipitation	10.6	
	DEAE-Cellulose	Ion-exchange chromatography	7.48	
	Sephadex G-300	Gel filtration chromatography	11.53	
Escherichia coli	Ni-NTA Affinity Chromatography	Affinity Chromatography	188	[21]
Escherichia coli	Sephacryl S-200	Gel filtration chromatography	141.3	[9]
	DEAE-Sepharose	Ion-exchange chromatography	312.8	
Escherichia coli	Ion-exchange chromatography using SP-Sepharose FF matrix	Ion-exchange chromatography	200	[22]
Escherichia coli	DEAE-Ion exchange chromatography	Ion-exchange chromatography	160	[23]
	Gel filtration chromatography	Gel filtration chromatography	190	

L-asparaginase can be isolated from plants and microorganisms. *Escherichia coli* is one of the microorganisms that can produce L-asparaginase with the greatest specific enzyme activity of 312.8 U/mg.[9] L-asparaginase obtained from *Escherichia coli* can be used as a first-line treatment for leukemia.[4] Determination of the specific enzyme activity of L-asparaginase from *Escherichia coli* can be carried out by an enzyme purification process.[9] Based on several scientific articles literature, there is a purification method to determine the specific enzyme activity of L-asparaginase from *Escherichia coli* including precipitation,[20] ion-exchange chromatography,[22] affinity chromatography,[21] and gel filtration

chromatography[23] with different specific enzyme activity results.

1. Precipitation

In this purification method, the salting-out method is carried out by adding ammonium sulfate salt to the protein. At a high salt concentration, the ionic strength in the salt will also be higher so that the salt will bind to water molecules. Ammonium sulfate is a salt that is often used for enzyme purification processes, because it has high solubility, has effective precipitation, and has high stability.[24] Protein solubility can be affected by the addition of high concentrations of salt. This happens because of the electric charge on the protein that can attract proteins and water molecules. Enzyme purification with the precipitation method using ammonium sulfate concentration of 80 % has a specific enzyme activity of 10.6 U/mg.[20]

2. Ion-exchange chromatography

Enzyme purification by ion-exchange chromatography (IEC) has the principle of separating proteins based on their surface ionic charge, using a modified resin with positive or negative chemical groups.[19] One of the advantages of ion-exchange chromatography is that the purification process is faster and can remove protein aggregates well. Based on the literature on enzyme purification using DEAE Sepharose ion-exchange chromatography, the specific enzyme activity was 312.8 U/mg,[9] enzyme purification by ion-exchange chromatography with SP-Sepharose FF matrix had a specific enzyme activity of 200 U/mg,[22] enzyme purification by ion-exchange chromatography with DEAE-Cellulose matrix has a specific enzyme activity of 7.48 U/mg.[20] All three are purification methods with ion chromatography which have different matrices and polymers, besides that they have different procedures so that the specific enzyme activities obtained are different. In DEAE Sepharose ion-exchange chromatography, rAS PG is expressed in *Escherichia coli* BL21(DE3) to Purified rAS PG, then 0.7 grams of cells from 100 ml of culture in LB medium (Lactose broth) were harvested by centrifugation at 8000 rpm at 40 °C for 5 minutes, and resuspended in 8 mL of 50 mM tris HCl buffer pH 8.6 then sonicated and centrifuged at 1200 rpm at 40 °C for 15 minutes.[9]

Enzyme purification by ion-exchange chromatography with SP-Sepharose FF matrix was carried out using of a BPG XK 16/20 column used for rhASP purification, purification was carried out at temperatures below 150C, then the concentrated solution obtained as much as 2 mL was inserted into the ion exchange column containing the SP-Sepharose FF matrix then the matrix was equilibrated with 10 column volumes of buffer containing 25 mM sodium

phosphate, 0.01% tween-20 at pH 5.2. After equilibration, the shASP protein was eluted linearly by providing a sodium phosphate buffer containing 50 mM sodium phosphate, 0.1% EDTA, and 1 M NaCl. The entire purification process was carried out under a constant flow rate of 2 ml/min.[22]

Enzyme purification by ion-exchange chromatography using DEAE-Cellulose matrix was carried out using of the previously purified enzyme fraction being applied to a DEAE-Cellulose column (1.5x80 cm) which was previously equalized with 0.01 M Tris HCl pH 8.0 at a flow rate of 1 ml/ minute. Then the fractions were collected and examined for enzyme activity and protein content.[20] In addition to different procedures, DEAE-Sepharose and DEAE-Cellulose matrices have differences, where the DEAE-Cellulose matrix has low capacity and has poor flow properties due to its irregular shape, while DEAE-Sepharose has better flow properties and capacity to macromolecules so that the specific enzyme activity they produce is different.[25]

3. Ni-NTA resin affinity chromatography

Affinity chromatography is a separation and purification technique for most biological molecules based on the chemical composition of these molecules, the basis of separation in affinity chromatography is the binding of protein molecules that have specific affinities with certain ligands. This technique is based on specific interactions between biological materials such as enzyme-substrate, enzyme-inhibitor, antigen-antibody.[26]

Purification using Ni-NTA resin affinity chromatography was carried out using of diluted protein used for purification using Ni-NTA resin affinity chromatography. The ready-to-use resin was equilibrated by adding a buffer containing 50 mM potassium phosphate, pH 7.8, 200 mM NaCl, 0.5 mM PMSF, and 20 mM imidazole). Then washed with 60 ml of equilibrium buffer consisting of 50 mM phosphate buffer, pH 7.8, 200 mM NaCl, 0.5 mM PMSF, and 20 mM imidazole. Proteins were eluted with 50 mM phosphate buffer elution buffer, pH 8.0, containing 250 mM imidazole and 1 mM PMSF. Fractions of 1 ml each were collected and analyzed by SDS-PAGE and protein concentration was measured using the Bradford stain method with BSA as standard. The fraction containing recombinant asparaginase was collected and dialyzed against 50 mM Tris-HCl, pH 8.0.[21] In principle, Ni-NTA resin containing nickel (Ni^{2+}) will bind to proteins that have 6x-His tag fusion and these bonds can be eluted using imidazole with high concentrations, this aims to release proteins containing 6x-His tag bound to Ni^{2+} ions. Purification using affinity chromatography of Ni-

NTA resin obtained a specific enzyme activity of 188 U/mg and the molecular weight obtained which was analyzed using SDS-PAGE was 141 kDa.[21]

4. Gel filtration chromatography

Gel filtration chromatography is a protein purification method based on differences in molecular size which is closely related to the molecular weight of the protein which has units (kDa). The chromatography will be faster. The purification process is carried out using a porous matrix packed inside the column and surrounded by solvent. Based on the literature the type of gel used is Sephadex G-300[20] and sephacryl S-200.[9] The purification procedure was carried out in multi-steps, before purification of L-asparaginase from *Escherichia coli* using Sephadex-300 gel filtration chromatography, first purification using ammonium sulfate, ion chromatography with DEAE-Cellulose. Where the elution profile of the most active fraction was loaded on the Sephadex G-300 column and obtained a specific enzyme activity of 11.53 U/mg.[20] Enzyme purification was carried out using the gel filtration method with Sephacryl S-200 which obtained a specific enzyme activity of 141.3 U/mg.[9]

In addition to the level of purity, enzymes have characteristics including determination of molecular weight, optimum temperature and optimum pH, which differ depending on the microorganism where the enzyme is produced. The L-asparaginase obtained from *Escherichia coli* has a molecular weight of 34 kDa-40 kDa obtained using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method, which is an electrophoretic method to separate proteins according to their molecular weight. The technique was carried out using a polyacrylamide gel containing Sodium Dodecyl Sulphate (SDS).[23] Then determine the optimum pH and temperature for L-asparaginase obtained from *Escherichia coli*. Based on some literature the optimum pH and temperature obtained were 8.5 and 500 C. Lakshmi *et al* (2015) in their in vivo research reported that L-asparaginase was effective in killing leukemia cells[27] with the mechanism of action of hydrolyzing L-asparagine into aspartate and ammonia by the L-asparaginase enzyme, so that tumor growth is inhibited due to a lack of asparaginase amino acid supply **(Figure.1.).**[20] In **Figure .1.** illustrated that tumor growth was inhibited due to the presence of the enzyme L-asparaginase which converts L-asparagine into aspartate and ammonia which resulted in the absence of nutrients to support the growth of tumor cells.

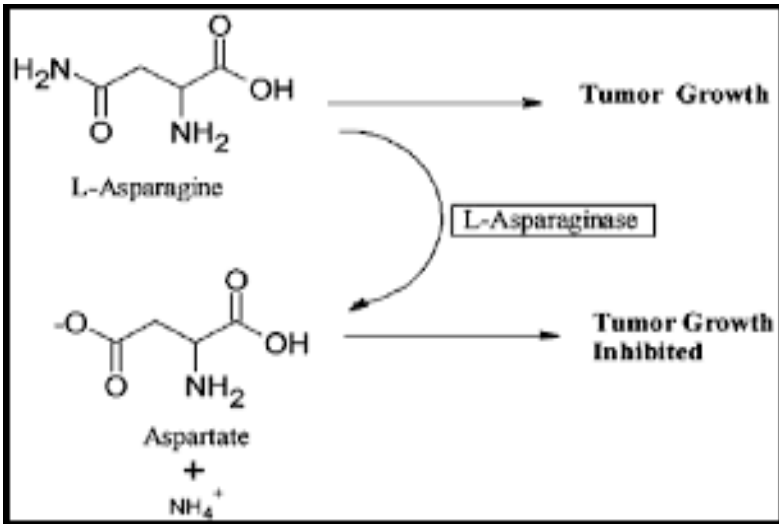

Figure 1: Illustration of the mechanism of L-asparaginase on tumor growth.[20]

CONCLUSION

Based on the results of the review of scientific articles obtained, there are several methods of purification of the L-asparaginase enzyme from *Escherichia coli* including precipitation, ion-exchange chromatography, affinity chromatography, and gel filtration chromatography. The greatest specific enzyme activity was using the ion-exchange purification method with DEAE-Sepharose matrix with a specific enzyme activity of 312 U/mg.

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