

Tongkat Ali Extraction using Hollow Fiber Membranes Modified by Negatively Charged-modifying Marcromolecules

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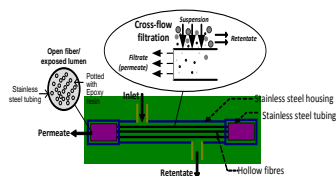
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Graphical abstract



Abstract

Eurycoma longifolia Jack is an herbal medicinal plant popularly recognized as 'Tongkat Ali.' The plant parts have been traditionally used for its antimalarial, aphrodisiac, anti-diabetic, antimicrobial and anti-pyretic activities, which have also been proved scientifically. This study attempt to isolate and concentrate the targeted 4.3 kDa peptide fraction from the Tongkat Ali water extracts which consist of many other fractions of peptides, proteins and phytochemicals by membrane separation. The hollow fiber membranes made of Polyethersulfone (PES) were fabricated in-house using phase inversion technique with synthesized Charged-Surface Modifying Macromolecules (cSMM) which anticipated by the end-capped group of cSMM namely Hydroxybenzene carboxylate (HBC). The influence of stock feed concentration and system flow rate were investigated in this work. The results obtained showed that the permeate is 10 times concentrated than the actual overall extract with linear influence on protein permeate concentration with increasing feed concentration. Whereas the flow rate of the feed stream has contribute to the flow rate and the concentration of the permeate stream an increased protein concentration by 5 % with the doubled feed flow rate.

Keywords: Negatively charge membrane; surface modifying macromolecules (SMM); polyethersulfone hollow fiber membrane

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1.0 INTRODUCTION

Eurycoma longifolia or locally known as Tongkat Ali is a traditional herb that is sought after the benefits of its pharmacological properties from its plant parts especially its root [1]. This herbal plant consists mainly of quassinoids, squalene derivatives, biophenyl neo lignans, tricullane-type triterpenes, canthine-6-one alkaloids and carboline alkaloids—though some stated α -carboline while some stated β -carboline [2, 3]. It is known for its anti-malarial, anti-pyretic and aphrodisiac effects [3, 4, 5]. Talbott *et al.* also found that Tongkat Ali has other benefits in reducing stress and improving psychological mood state in humans [2].

Traditionally, Tongkat Ali is extracted by boiling its plant parts (usually the tap root) in water [4]. In herbal and phytochemical processing, the challenges to increase product yield while maintaining overall process at reasonable economics and to produce standardized extracts with the active ingredients in the desired concentration profile are the major concerns of study.

On the other side, membrane technology emerged as one of the most promising options for liquid purification [6]. According to Rana *et al.*, membrane has become the choice for separation and filtration because of its ability in disinfecting

water without chemical thus, avoiding formation of toxic byproducts [7]. Yet in membrane processes, the success of any separation system involving membrane depends on the quality and suitability of the membrane incorporated in the system. In Tongkat Ali extraction system, membrane is used as filter to separate the Tongkat Ali compounds that was already extracted via any extraction method (water extraction or ethanol extraction) to the desired protein fraction.

This paper concentrates on the utilization of membrane separation for Tongkat Ali extraction to isolate and concentrate the targeted 4.3 kDa peptide fraction from the Tongkat Ali water extract.

2.0 EXPERIMENTAL

2.1 Tongkat Ali Extraction

There are quite a number of extraction methods that can be conducted to extract the Tongkat Ali. According to Mohamad *et al.*, the ideal extraction method should be non-destructive, quantitative as well as time efficient [9]. Hot water extraction of Tongkat Ali is still used widely [1, 5, 8, 9] while there is also ethanol extraction [1, 2, 10]. Other researchers also used solvent

extraction where alcohol and water are mixed with solvent such as chloroform and silica [3, 4, 11].

In this study, the Tongkat Ali is extracted using water extraction method. The boiled extract was then run through a filter system to obtain the desired 4.3 kDa peptide fraction. Hollow fiber membrane was utilized as the filter media in the filtration system.

2.2 Peptide Separation from Tongkat Ali Extract

Tongkat Ali extract contains many fractions of peptides, proteins and phytochemicals. The focus of this study is to further extract 4.3 kDa peptide from a water-extracted Tongkat Ali. Membrane are used to separate the targeted peptide from the Tongkat Ali extract. Affinity membrane was first proposed to be used as well ceramic membrane cartridges and hollow fiber membrane. These membranes were compared in terms of separation and the best one is further used throughout the study.

2.3 Hollow Fiber Membranes and Separation Permeation Tests

Hollow fiber membranes were fabricated in the laboratory using a simple phase inversion technique. The hollow fiber membranes were spun at wet/wet condition using water as coagulant both in the coagulation tank and in the bore fluid. Prior to spinning, a spinning dope consisting of Polyethersulfone (PES), water (deionised), cSMM and N-methyl-2-pyrrolidinone was prepared by the procedure described elsewhere [12] to produce an asymmetric hollow fiber membrane. Table 1 shows the fabrication process and spinning conditions of hollow fiber membranes used in this work. The additive of cSMMs is the oligomeric glycol polymers synthesized by polyurethane chemistry and tailored with end groups. Hypothetically, the charges were contributed by the end-capped group of cSMM which were carboxylate ion¹³ as shown in Table 1.

Table 1 Fabricated hollow fiber

Technical fabrication process	Membranes: PD1-5
Dope formulation (wt%)	PES (22)
Additive (cSMM)	Hydroxybenzene carboxylate
	<chem>Oc1ccc(cc1)C(=O)[O-]</chem>
<u>Spinning conditions:</u>	
Dope flow rate:	3.0 cm ³ /min
Bore fluid composition:	Distilled water
Ratio Outer Diameter: Inside Diameter	ID/OD 0.3/0.6
Air gap distance	5 cm

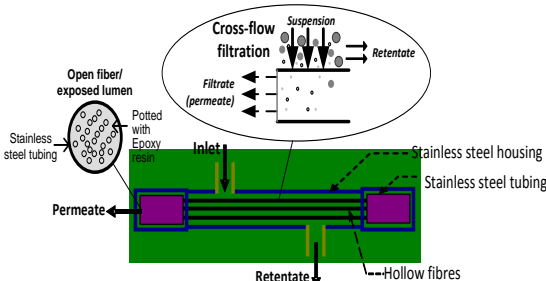


Figure 1 Schematic diagram of hollow fibre membrane module at the cross-flow filtration process

Water permeability test was conducted for the fabricated hollow fiber membrane at cross-flow filtration as illustrated in Figure 1. Then, the protein separation properties of Tongkat Ali were further studied focusing at the effect of feed concentration and system flow rate. Controlling these parameters at the very initial stage contributed to a well enhanced final outcome of the membrane separated compounds.

3.0 RESULTS AND DISCUSSION

The results of filtration performance are shown in Figure 2. The permeate flux versus the applied pressure for all the three membranes obtained a linear profile. The slope of the lines represents the water permeability value for PD1-5 hollow fiber membrane at pressure range of 2.5-8.5 Bar. The normalized pure water fluxes for PD1-5 hollow fiber membrane used in this work observed at 5.3 L/m².h.Bar.

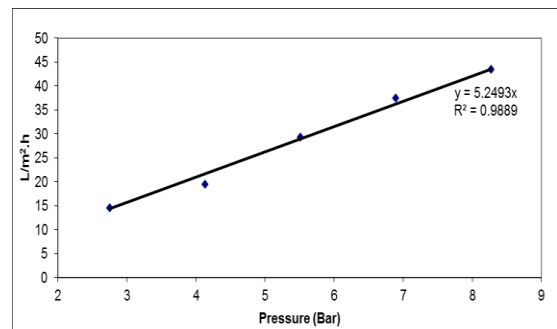


Figure 2 Pure water permeability (L/m².h) of HF PD1-5 pressure of at 2.5 to 8.5 Bar

The influences of feed concentration are shown in Figure 3. Concentration influence on separation efficiency was determined by using three different initial stock concentration of Tongkat Ali extract. The three initial concentrations were 0.005, 0.0075 and 0.01 g/mL as presented in Figure 3. The 0.01 g/mL concentration was fixed as the maximum concentration for this study as this concentration is almost 10 times concentrated than the actual overall extract. Absorbance measurement for concentration determination via UV/Vis instrument also limits this as the absorbance touched the critical photometric range. The analyzed permeate and retentate is of the second batch after flushing out the first batch of elutant which may contain small amount of water and subsequently provide an altered reading.

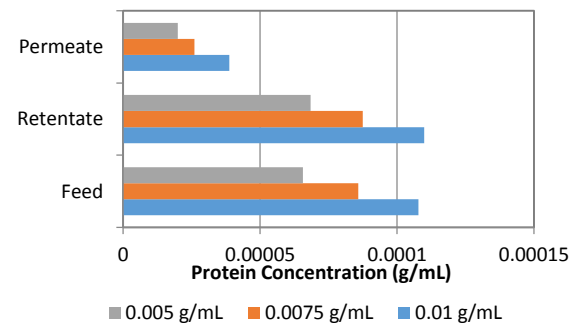


Figure 3 Protein concentration of all three streams based on varied stock feed concentration

From Figure 3, it was observed that there is direct linear influence on protein permeate concentration with increasing feed concentration. However, a slight difference by 6% between the permeate of the 0.0075 and 0.01 g/mL stock concentration indicates that these observation needs further investigation.

The flow rate of the feed stream was believed to contribute to the flow rate and the concentration of the permeate stream in a proportional way. The stock feed concentration was fixed at 0.01 g/mL at room temperature while flow rate varied as presented in Figure 4. As expected the permeate stream indicated an increase protein concentration by 5% with the doubled feed flow rate.

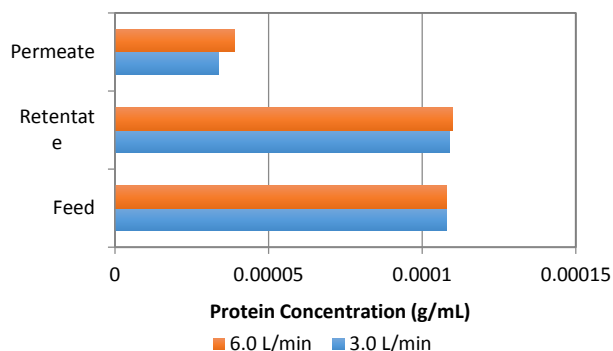


Figure 4 Effect of flow rate on the protein concentration of permeate stream

Experimental design using Response Surface Model (RSM) was analyzed for protein content and respective models describing the protein concentration in all streams was constructed. Overall observation on the models indicates protein content also have very similar models with their overall feed and overall retentate concentration. Figure 5 represents both the protein concentration model of feed and retentate streams.

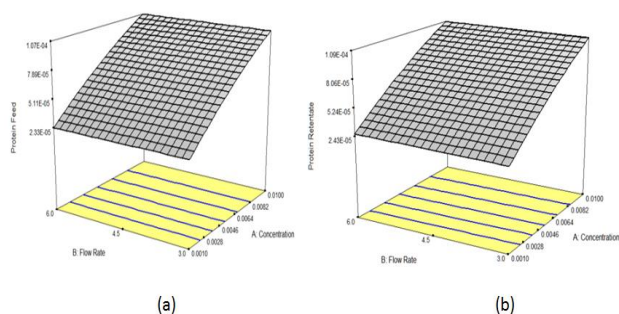


Figure 5 Protein concentration RSM of (a) feed and (b) retentate stream

From Figure 5, almost up to 2% gradual increase in protein concentration was observed in the retentate stream up to the 0.0075 g/mL stock feed concentration separation. This indicated most of the water content in the feed stock solution together with some other non-protein compounds was able to separate out resulting in slightly higher protein concentration at the retentate stream.

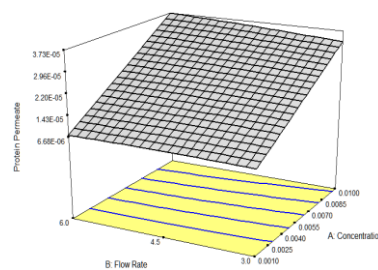


Figure 6 RSM of protein concentration for permeate stream

On the other hand, the sudden oppositeness with 1.8% of higher protein concentration in the stock feed stream compared to retentate stream at the highest concentration, resolves that higher concentration able to promote greater separation on the proteins molecules rather than the water molecules. The model of permeate stream for protein concentration shows a very clear linear relationship of concentration and flow rate enhancement effect towards the protein separation profile as in Figure 6.

Even though the separated protein compound is in very low amount but the significance of the experimental parameter on protein separation is very clear on the above response surface model. Highest stock feed concentration did able to produce greater concentration of permeate stream with significant effect of increased flow rate.

Here, it seems like protein permeation need further separation parameters with stock feed concentrations greater than 0.01 g/mL and also further higher flow rates as the model looks very much linear indicating no optimum points. Hence, after considering the performance of the gear pump on greater concentration of stock Tongkat Ali extract and also the capacity of the hollow fiber membrane module, thus further study beyond this point is not possible.

4.0 CONCLUSION

The attempt to isolate and concentrate the targeted 4.3 kDa peptide fraction from the Tongkat Ali water extract which consist of many other fractions of peptides, proteins and phytochemicals were conducted with fabricated Polyethersulfone (PES) hollow fiber membrane modified with charged surface modifying macromolecule (cSMM). It was concluded that in-house fabricated hollow fiber membrane blend with synthesized cSMM which anticipated by the end-capped group of cSMM namely Hydroxybenzene carboxylate (HBC) has successfully extracted Tongkat Ali Peptide. It was observed that the permeates were 10 times concentrated than the actual overall extract with linear influence on protein permeate concentration with increasing feed concentration. In addition, most of the water content in the feed with some other non-protein compounds was able to separate out resulting in slightly higher protein concentration at the retentate stream at rate of 2 % gradual increase in protein concentration in the retentate stream.

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