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Aqueous Two-Phase Flotation for Extraction of Andrographolide from Andrographis Paniculata

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This paper was presented about the extraction of bioactive ingredient that is beneficial towards skin and human health from herbs. Andrographis Paniculata was used for this paper to undergo optimisation of extraction method by modern and novel extraction technique which is aqueous two-phase flotation (ATPF). ATPF extraction is rapid and has simple extraction procedures, resulting in achieving the target yield in least time and energy saving. Under comparison between sonication, soxhlet, aqueous two-phase system (ATPS), and ATPF extraction techniques, ATPF had been chosen as the more effective extraction method for Andrographis Paniculata. The optimised ATPF system was able to yield 5.85 % of Andrographolide with the ATPF's operating condition set to 38.96 wt% methanol as top phase, 12 wt% di-potassium hydrogen phosphate as bottom phase, 30 min of flotation time, 50 mL/min nitrogen gas flowrate, and 0.75 g of crude load at ambient temperature (25 ± 1 ºC).

1. Introduction

Plants are important sources of medicines, especially, in developing countries such as Malaysia where in most cases plant-base products are often used for medication and healthcare. The World Health Organization (WHO) estimated that around 80 % of the world's population relied on medical plants as their primary healthcare source. Although modern medicine may be available in most developing countries, the popularity towards herbal medicines are still high especially for historical and cultural reasons (World Health Organization, 1999). Few herbal species that provide medicinal herbs have been scientifically assessed for their medicinal use. Most of these herbal plants have very little knowledge and data about their active ingredients and the amount of active ingredients present in them. Andrographis Paniculata is an herbal plant that contains bioactive components that are beneficial to human health and skin.

Andrographis Paniculata is an herbal plant and it is commonly used in traditional Chinese medicine for its detoxifying, antisepsis and anti-inflammation effect to humans. Apart from that, Andrographis Paniculata also has other treatment abilities which include anti-microbial activity, anti-allergic activity and anti-oxidant activity (Okhuarobo et al., 2014). The bioactive component that gives this ability is Andrographolide which is also the abundant constituent in this plant (Jayamohan et al., 2013). In the future, it is expected that Andrographis Paniculata would give anti-HIV and anti-cancer properties and it is currently being studied using cultivated cells (Niranjan et al., 2010).

Herbal plant extraction is a fairly common process used to extract bioactive components which are useful for medicinal use. Extraction techniques can be categories into two groups which are traditional extraction methods and technological (modern) extraction methods. Traditional extraction techniques include maceration extraction, decoction extraction, percolation extraction, and infusion extraction method whereas the modern extraction includes sonication (ultrasound-assisted extraction), Soxhlet extraction, aqueous two-phase system (ATPS), aqueous two-phase flotation (ATPF) and others (Vasisht, 2008). ATPS has been used in herbs extraction, as reported in the literature (Coêlhoa et al., 2015).

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ATPF is the enhanced method from ATPS. ATPF is a flotation technique the employed a constant flow of gas stream (nitrogen gas or others) ascending a column of immiscible liquid mixture. The target component is then absorbed on the surface of gas bubble and travel through the column. As a result, a hydrophobic component at the bottom of the column could be easily transported to the immiscible liquid layer which is usually is an organic liquid layer and to be dissolved. (Han et al., 2014). Besides, it is also believed that by incorporating ATPF in herbal extraction, the surface active components can be extracted easily through ATPF. In addition, the main advantages for ATPF technique is use of low toxicity forming chemicals, biocompatibility, high product yield, low cost due to lower chemical usage, price, and scale up potential compared to traditional extraction method. (Show et al., 2013)
Extraction of bioactive components from herbs is commonly being carried out in industries through maceration with agitation. It is believed that maceration is a slow extraction process, but with the aid of constant agitation, the overall mass transfer of the bioactive component will increase. However, the efficiency and profit of herbal extraction can be enhanced if a more rapid extraction operation such as ATPF was used. However, the quantification and optimisation of bioactive components present in *Andrographis Paniculata* herbs is rarely done. Therefore, in this research, optimisation of ATPF was done to give andrographolide high extraction yield. ATPF extraction optimisation was undergoing several parameters which include flotation time, nitrogen gas flowrate, top phase concentration, bottom phase concentration and mass of herbs to obtain the highest yield possible.

2. Materials and methods

2.1 Materials

Andrographis Paniculata was obtained from local plantation in Pahang, Malaysia. Andrographolide (purity = 98 %), methanol and di-potassium hydrogen phosphate (K_2HPO_4) in analytical grade were purchased from Sigma Aldrich.

2.2 Extraction technique

Before the experiment started, the Andrographis Paniculata was grinded into powdered form by using centrifuge mill before being stored in re-sealable packets in a secured cabinet. A glass column with sintered glass disc G4 porosity was set up as shown in Figure 1 and the content inside the tube was incorporated with purified nitrogen gas as an inert gas for bubbling from the bottom of the glass column. Firstly, 50 g ATPF system with 15 wt% K_2HPO_4 salt solution, 0.1 g of powdered Andrographis Paniculata, and 50 wt% methanol solution were filled one after another in a vertical column tube as shown in Figure 1. Then the top of the glass column was sealed with parafilm to minimize methanol's evaporation, the experiment was set for 30 min with 50 mL/min continuous nitrogen gas flow. After 30 min, all the mixture from the glass column was transferred to a 50 mL of centrifugal tube for settling to allow phase separation prior to quantification.
ATPF Optimisation was carried out to maximise the yield percentage of Andrographolides from Andrographis Paniculata. The ATPF parameters such as flotation time, flowrate of nitrogen gas, top phase concentration (methanol-rich phase concentration), bottom phase concentration (salt-rich phase concentration) and crude load were chosen to optimize. The initial ATPF parameters were preset as gas flotation time at 30 min, 50 mL/min nitrogen gas flowrate, 48.96 wt% methanol solution, 15 wt% K_2HPO_4 and 0.5 g of herb powder. The optimisation approach used was one-variable-at-a-time, so the initial operating conditions were to be maintained until that the mentioned operating condition was optimised. One variable at a time optimisation approach is still an acceptable approach for most of the preliminary studies in Bioseparation Engineering, as in the literature (Show et al., 2013). This approach was adopted to study the effect of the variable change on the extraction performance.

2.3 Analytical method

The UV-Visible spectrophotometer with model Shimadzu UV-1800 was used throughout the experiment to quantify the extracts from Andrographis Paniculata. Calibration curve was created by incorporating the reference standard Andrographolides. By using pure methanol as reference, measure the amount of Andrographolides being extracted in term of absorbance at 230 nm (Sharma and Sharma, 2013). The yield of extracted Andrographolides from Andrographis Paniculata could be obtained using Eq(1).

$$Yield\,(\%) = \frac{m_A}{m_H} \times 100\% \qquad (1)$$

where m_A the mass of Andrographolides being extracted (mg) is, m_H is the mass of herbs used (mg).

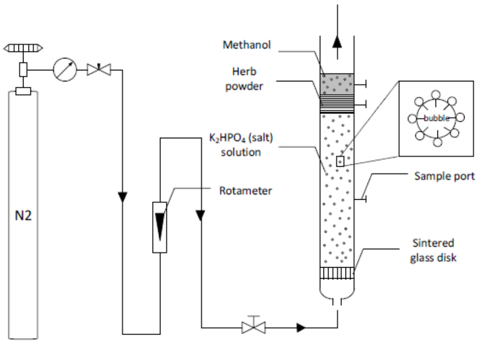


Figure 1: Schematic diagram of ATPF process

3. Results and discussion

3.1 Solubility of andrographolide

Solvent study and selection was carried out in order to identify the most efficient solvent to extract Andrographolide. According to Figure 2, Andrographolide consists of three hydroxyl groups which mean that it is a polar component by nature. However, it is well known that Andrographolide is sparingly soluble in water because of its long alkyl chains. As a result, the hydrophobic property of the alkyl chains dominates and outweighs the polar property of the hydroxyl groups, making it to be more hydrophobic than hydrophilic. So, in order to extract Andrographolide effectively, the solvent must be a hydrophilic organic solvent such as methanol, ethanol, acetone, chloroform or others. In fact, methanol is an hydrophilic organic solvent that has the high polarity and managed to extract the highest amount of Andrographolide out of the other hydrophilic organic solvents (Jadhao et al., 2014). Thus, methanol was used as extraction solvent.

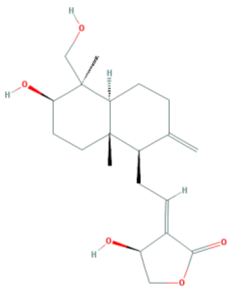
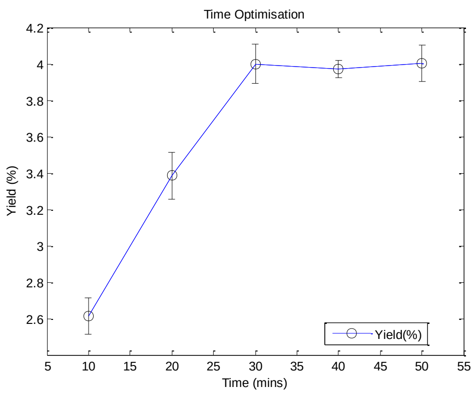


Figure 2: Chemical Structure of Andrographolide (PubChem Compound Database; CID=5318517, n.d.)

3.2 Effect of flotation time and N_2 flowrate

The time parameter optimisation study was done to determine the effect of the flotation time towards the amount of andrographolide recovered. As shown in Figure 4, the percentage andrographolide recovery increased drastically from 10 min to 30 min where the recovery was peaked at the 30 min mark with the andrographolide recovery of 3.9995 %. In addition, it is believed that as the flotation time increased, the interaction between the N_2 bubbles and the andrographolide was completed. Thus, the time was long enough to allow the maximum transfer of andrographolide via N_2 bubbles into the top phase. After the 30 min's mark, the percentage andrographolide recovery decreased slightly and increased back to the peak point at 50 min. In fact, it is believed that saturation of andrographolide on the top phase was ensured after the 30 min mark, and further increasing the flotation time would not be able to increase the amount of andrographolide recovery anymore. So, in order to save time and reduce the usage of N_2 gas at the same time, 30 min of flotation time was chosen as the operating parameter for time.

After the time optimisation was completed, the optimum time parameter was incorporated and maintained constant throughout the rest of optimisation study. The same will also be done to other parameters. The N_2 flowrate parameter was done within the range of 30 mL/min to 70 mL/min with an interval of 10 mL/min. The results for this optimisation study are presented in Figure 4 with the optimum point remained at 50 mL/min with 4 % Andrographolide being yielded. Gas flow rate plays an important role for ATPF separation reach equilibrium. Generally, high flow rate increases increase gas-liquid interface area by creating more bubbles and accelerates mass transfer, reduce time for separation equilibrium reach. As shown in Figure 4, gas flowrate of 50 mL/min reached equilibrium approximately 30 min with percentage extraction of 4.00 ± 0.11 %.



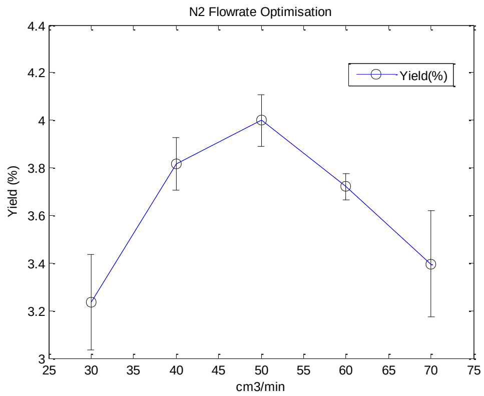


Figure 3: Flotation time parameter optimisation   *Figure 4: N_2 flowrate parameter optimisation*

However, the position of rising bubbles at the interface is determined by the balance of floating force (upwards) and interface tension (downwards) based on reported literature recently (Show et al., 2013). Bubbles need to be large enough, so they could overcome the interfacial tension and reach methanol phase at herb powder interface. Along with the burst of bubbles, Andrographolide with three hydroxyl group which is hydrophilic attached to the water at bubble interface were released and dissolved. As bottom phase consist of large amount of water which makes the interfacial tension much weaker. In comparison, the interfacial tension between upper top phases, herb powder phase and methanol phase, is much larger due to the solid phase of herb powders. Therefore, slow gas flowrate of 30 and 40 mL/min shown in Figure 3 result in lower percentage yield of 3.24 ± 0.2 % and 3.82 ± 0.11 % due to slower mass transfer rate with smaller bubbles interfacial area. Nonetheless, high nitrogen flowrate of 60 and 70 mL/min with percentage yield of 3.72 ± 0.06 % and 3.40 ± 0.22 % does not make the extraction better due to the foam accumulation at herb powder phase that cause the bubble burst and trapped at bottom phase. Besides, it is believed that the water – methanol interface of the ATPF system was disrupted drastically until the extracts will not be able to reach the top phase successfully when the flowrate was higher than 50 mL/min (Han et al., 2014). Therefore, 50 mL/min of gas flowrate and 30 min flotation were a good choice to obtain optimum result.

3.3 Effect of Top Phase (Methanol) Concentration

In ATPF, two phase region only occur within certain methanol and salt concentration. Thus, it is important to find out the optimum methanol concentration for extraction. By changing methanol concentration while maintain salt concentration, the volume ratio between methanol phase and salt phase greatly influenced. Volume ratio affect distribution ratio of bio-active component as high volume ratio (large methanol concentration) cause low percentage yield of Andrographolide. The high methanol concentration lead to stronger interfacial tension of the methanol-salt interface and thus more gas bubbles become bursting before manage to pass through the interface, and in turn lead to decrease in the percentage yield. From Figure 5, we can observe the drastically decrease of percentage yield from 4.63 ± 0.07 % to 3.53 ± 0.09 % when methanol concentration increase from 38.959 wt% to 58.959 wt%. However, volume ratio cannot be too low due to the decreasing in methanol volume cause the equilibrium dissolution capacity of Andrographolide in methanol reach. Thus, the extra Andrographolide have to dissolve in water than have weaker solubility compared to methanol. Figure 5 shows the decrease in methanol concentration from 38.959 wt% to 33.959 wt% result in percentage yield reduction from 4.63 ± 0.07 % to 4.36 ± 0.005 % that

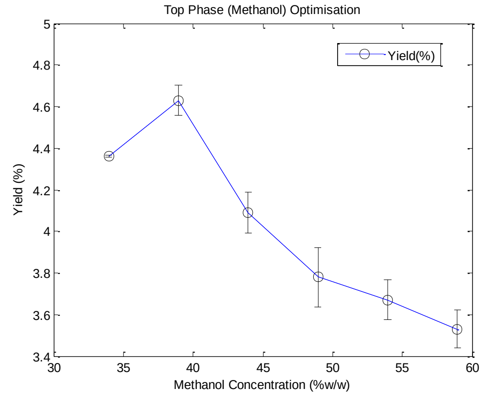


Figure 5: Top Phase Parameter Optimisation

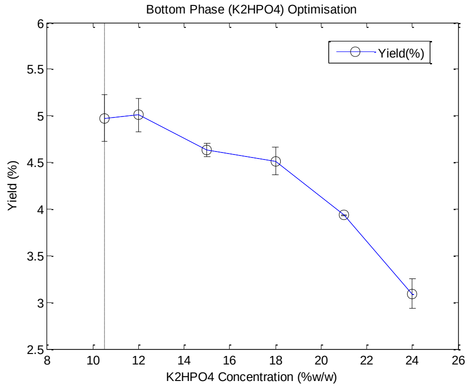


Figure 6: Bottom Phase Parameter Optimisation

satisfies the above statement. Therefore, the optimum methanol concentration obtained was 38.959 wt% with percentage extraction of 4.63 ± 0.07 %.

3.4 Effect of Bottom Phase (K_2HPO_4) Concentration

Aqueous salt phase needs enough amount of salt to maintain phase separation. Phase separation of aqueous two phase system occurred due to the competitive hydration of salt and methanol. The influence of salt concentration to percentage yield of Andrographolide can be explained as “salting-out” effect. The increase in potassium phosphate dibasic concentration causes the decrease of free water amount in aqueous phase result in increase of volume ratio. The reduction of free water amount causes andrographolide percentage yield to decrease as the hydrated ions on the bubble’s interface increases. Thus, the interfacial contact area of Andrographolide with free water molecules decrease causes lesser transportation to methanol phase. Besides, the decrease in water amount also reduces the interfacial tension of aqueous phase that further trapped Andrographolide in salt solution whereas the reduction in salt concentration aid extraction vice versa.

Figure 6 shows decrease in percentage extraction acutely from 5.00 ± 0.18 % to 3.09 ± 0.16 % when salt concentration increase from 12 wt% to 24 wt%. However, there is no improvement in percentage yield when salt concentration decreases from 12 wt% to 10.5 wt% as dissolution capacity of methanol reach. Thus, Andrographolide forced to dissolve in water that has lower affinity compared to methanol. Therefore, salt concentration of 12 wt% result in optimum percentage yield of 5.00 ± 0.18 %.

3.5 Effect of Crude Load

When mass of A.P. increase, thicker herb powder’s phase was observed result in stronger interfacial tension for bubble to reach methanol phase. Thus, the bubbles will break in herb powder’s interface cause percentage yield to decrease. Figure 7 shows the decrease in percentage yield from 5.85 ± 0.33 % to 4.70 ± 0.16 % when A.P. mass increase from 0.75 g to 1.05 g. However, the reduction of herb mass from 0.75g to 0.25 g also shows decrease in percentage yield from 5.85 ± 0.33 % to 4.79 ± 0.16 %. It is because lesser herb powder cause interfacial tension becomes weaker lead to large amount of bubbles causing foam accumulation. It decrease interfacial contact area of Andrographolide and stopped some fraction of bubbles to reach methanol phase. Therefore, the optimum A.P. mass was 0.75 g with percentage yield of 5.85 ± 0.33 %.

4. Conclusions

Herbal extraction is a simple and a straight forward process that can be done via any combination of solvent and extraction method used, and the openness of those methods are at the highest, which it can be done by anyone. However, sustainability issue was faced in those conventional due to the fact that most of those methods employed too much usage of solvents in order to extract parts of the active ingredients presented in herbs with low efficiency and non-recyclable most of the time, and/or required intensive amount of energy in order to complete the extraction process. Therefore, in this study shows that out ATPS and ATPF, ATPF system was the superior extraction method which gives a higher yield of Andrographolide. Before optimising the ATPF extraction, the percentage yield was found to be 2.61 % and after optimizing the flotation time to 30 min, N_2 flowrate to 50 mL/min, methanol concentration to 38.96 wt% and K_2HPO_4 concentration to 12 wt% and mass of herbs to 0.75 g the percentage yield is 5.85 %.

Therefore this is a promising and novel extraction technique that can be applied for medicinal plant extraction and for pharmaceutical industries in the near future with the availability of suitable technology.

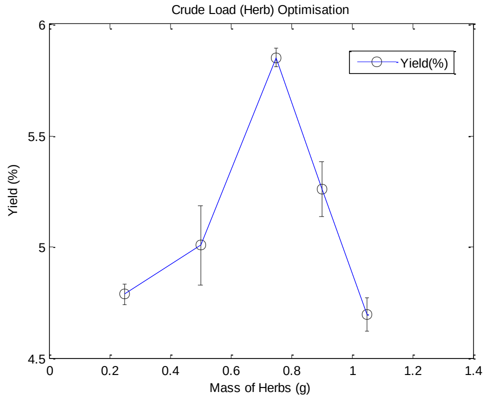


Figure 7: Crude Load Parameter Optimisation

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