Effect of Extraction Factors and Mass Transfer on Total Phenol Content and Antioxidant Activity of Wolffia Globosa L.

Chulawan Dorkmaingam*, Pimporn Polpech and Chutimon Satirapipathkul
Chemical Engineering Research Unit for Value Adding of Bioresources, Department of Chemical Engineering, Chulalongkorn University, Bangkok 10330, Thailand

Abstract: The objective of present work was assessing the optimization of a batch extraction process for phenolic compounds from Wolffia globosa. The effects of extraction temperature, solvent concentration, ratio of solvent to solid and mass transfer during extraction process on phenolic compound yield and antioxidant activity were investigated. The suitable condition for batch extraction were 70% (v/v) by using ethanol as solvent, extraction temperature at 50 °C, solvent to solid ratio of 20:1 (ml/g) and extraction time was 180 minutes, from which the yield of phenolic compounds was 40.90 mg. equivalent gallic acid / g weight was gained. Whereas the DPPH assay was 35 percent DPPH. The extraction results indicated that antioxidant power was highly correlated to the content of phenolic compounds. The mass transfer during extraction process was observed. As the result of study, effective diffusivity (D_{ab}) was maximum by increasing temperature to 50 °C which obtained the result of D_{ab} at 13.20 \times 10^{-6} m^2/s

Keywords: Wolffia globosa, Phenolic Compounds, Antioxidant, Extraction

1. Introduction

Most recently, attempts have been made to contend with the impressive performance of biomaterials for application in human health and perform as drug-like herbs, making it an essential topic in bioengineering fields. Effective argumentation of assorted phytochemicals for herbal cosmetics is increasing the value of raw materials that originate from rural areas. Such materials are naturally useful and do not have any negative side effects on human skin, which corresponds to people’s exigency at present.

Fig. 1: Wolffia Globosa Plant

Wolffia globosa L. (Lemmaceae), the smallest aquatic plant in the duckweed family, is found on hydrostatic water in regions of Thailand, Southeast Asia and China. It has become an increasing trend for direct utilization
in healthy/hygienic food for humans because it contains such high protein content (34-45%) in dry weight [1]. Wang Nini et al. [2] investigated the chemical constituents of W. globosa in terms of quantitative analysis. Their report showed that W. globosa is a newly noticeable fountainhead of bioactive compounds in the derivative of phenolic compounds.

T. Ozcan et al. [3] suggested that phenolic compounds have a significant role in health benefits, particularly due to attributes that act on antioxidant and antimicrobial activities as well as recognition that they provide anti-inflammation, anti-infective, and anti-disease benefits through neutralization mechanisms. Phenolic compounds are ubiquitous in a wide range of plants and generally divided into four groups including phenolic acids, flavonoids, stilbenes and tannins. The phenolic benefits on human health, which not only energize as an antiseptic, but also are commonly used as active ingredients in sunscreens, resulting in the effective absorbance of UV-B radiation between 280 and 315 nm.

The batch extraction process is typically utilized as a preliminary process for separation to gain a crude extract of bioactive compound from plant materials. The results from previous study show that optimized conditions have unique properties based on each material [4]. Typically, the key factors that potentially influence the rate and yield of extraction for phenolic compounds include solvent type, operation temperature, particle size and extraction time.

Typically, the mass transfer effect during the extraction process could be study. The extraction process also base on rate of bioactive compound dissolve to reach the equilibrium concentration in the solvent. Theoretically, four mass transfer steps are concerned but from previous study work the diffusion of the dissolved solute within the solid into the solvent is the rate limiting step (Gertenbach, 2001)[5]. The rate of diffusion of controlling step can be explained by Fick’s second law

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}
\]

Where C is the concentration of the solute, t is time, D is the diffusion coefficient or diffusivity, and x is the distance of diffusion. While process of extraction was operated. The mass transfer of the bioactive compound from plant particle to the solvent will effect on the phenolic composition yield. There are various models in the previous study that refer to the internal mass transfer (Aguilera and Stanley,1999)[6]. Diffusion within the particle is the controlling step of the extraction process. Typically, effective diffusion was determined in experimental data.

The research aims were, therefore, to study the optimize extraction phenolic compounds from W. Globosa by varying the key parameters which effect to yield of polyphenol that including extraction temperature, solvent concentration and ratio of solvent to solid. The effective diffusivity, \(D_{eff}\) was determined to estimate effect of internal mass transfer during extraction occur. The results of extraction yield were calculated and reported by total phenolic content (TPC) and antioxidant activity (DPPH percent scavenging activity) in term of Gallic acid equivalents (mg GAE/g) of W. Globosa and percent DPPH respectively.

2. Experiment

2.1. Material Preparation

W. globosa used in the research work was purchased from the Agricultural Organization in Nakhon Ratchasima, Thailand. Foline Ciocalteu’s reagent, gallic acid, sodium hydroxide, aluminum chloride anhydrous, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, ferric chloride anhydrous, trolox, were supported by research unit for value adding of bioresources Chulalongkorn University.
2.2. Preparation of Extraction

Fresh W. globosa was washed carefully with non-ionic water, after which it was freeze-dried at -20 °C. Then, the sample was ground to powder using a kitten-milling machine.

2.3. Extraction Method

The extraction method was performed on 1 g of Wolffia Globosa which was immersed in flasks containing ethanol by varying extraction factor that were ethanol concentration, extraction time and solvent to solid ratio. Then the suspensions were filtrated with Whatman No.42 filter paper, and the residue was washed with ethanol. The insoluble residue was discarded. The filtrate was evaporated in a water bath at 50 °C. The evaporated residues were used for the analysis of the total phenolic content (TPC), antioxidant activity (% DPPH Scavenging) and effective diffusivity (D\text{ab}).

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic compound content (TPC) of each sample extract, from which the various study conditions were assessed, used slight modifications of the Folin-Ciocalteu spectrometric method [7]. In brief, 20 μl of extract solution with proper dilutions were mixed with 100 μl of Folin-Ciocalteu reagent, which was kept as a solution in the dark at room temperature overnight. Subsequently, 80 μl of 7.5% sodium carbonate was added to the solution. Finally, the absorbance was determined by a microplate reader at a wavelength of 760 nm. The contents of the phenolic compound were reported as mg of gallic acid equivalent (GAE)/g weight of W. globosa.

2.5. Determination of Antioxidant Activity (% DPPH Scavenging)

The antioxidant activity of each sample extract, from which the various study conditions were assessed. The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picrylhydrazyl DPPH spectrophotometric assay, with some modifications from Mensor et al. (2001) [8]. Briefly, the samples 0.2 ml were mixed with a 0.20 mM DPPH ethanol solution for 2 ml. After incubation at room temperature in the dark for 30 min, the mixture was measured at the absorbance of 517 nm using a spectrophotometer. The standard curve was linear between 0.08 and 0.64 mM Trolox. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the following equation

\[
\%\text{DPPH} = 100 \times [1 - (A_e / A_d)]
\]

where  
A\text{e} is the absorbance of the DPPH solution with the extract added 
A\text{d} is the absorbance of the DPPH solution with nothing added. Weight of Wolffia Globosa

2.6. Determination of Effective Diffusivity (D\text{ab})

In this study work, the extraction of phenolic compounds from plant assumption same as previous research is that the diffusion within the particles of Wolffia globosa is the process that controls the extraction process. The diffusion process within the particle of the plant is based on the diffusion distance or particle size of porous. In case of the hole is more scatter solvent can penetrate longer distances and given the more effective diffusivity, D\text{ab} results. In order to gain the D\text{ab} in each condition logarithm of Y is plotted against time, a straight line should be obtained and the diffusivity can be examined from its slope [9]

\[
\ln Y = \ln \left( \frac{\delta}{\pi r^2} \right) - \frac{\pi^2 D\text{eff} t}{r^2}
\]

where  
Y is the ratio of C\text{ao}/C\text{as}.

C\text{ao}/C\text{as} is ratio of phenolic concentration at any time and phenolic compounds in the initial, t is operation time, r is the particle size of Wolffia globosa.
3. Result and Discussion

Phenolic extracts from W. globosa were prepared by batch extraction as an alternative method. Results are shown in Figure 3-9. Based on Figure 3, it was observed that there was a significant difference between TPC at extraction times between 0-180 minutes. After extraction time of 180 minutes, the phenolic yield increased slightly with no significant distinction. This trend could be explained in terms of mass transfer, which had the result from concentration gradient between the interfacial phase and bulk solution.

Fig. 3: Extraction yield at 0-240 minute by using ethanol concentration 30, 50, 70 and 95 percent

At the starting process, the large driving force caused an increase in effective diffusivity. Hence, the experiment results and economic point of view showed that W. globosa provided the optimum extraction time of 180 minutes assorted as the optimum extraction time for investigating other parameters.

Fig. 4: % DPPH assay scavenging at 240 minute minute by using ethanol concentration 30, 50, 70 and 95 percent

Based on Figure 4,5 increasing ethanol concentration to 70 percent brings about higher TPC and DPPH scavenging activity. Accordingly, this trend could suggest that most of the phenolic compounds in W. globosa had moderately polar characteristics. Moreover, higher ethanol concentration could cause to protein denaturation and lower phenolic yield.

The results illustrated in figure 5, show that the yields of phenolic compounds increased from 35.39 (GAE)/g wt of W.globosa.to 38.92 (GAE)/g wt of W.globosa.with increasing extraction temperatures from 25°C to 50°C.

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These results could be explained as a consequence of high temperature, which caused less viscosity and created higher effective diffusivity, corresponding with equation 4[9]. Nevertheless, solvent will be deprived and oxidation of the phenolic content will occur if the temperature is too high [10]. This results in the effect of yield increase temperature to 60 °C.

\[ D_{ab} \propto \frac{T}{\eta} \]  \hspace{1cm} (4)

where \( D_{ab} \) is effective diffusivity, \( T \) is operation temperature and \( \eta \) is viscosity

Fig. 5: Extraction yield at 240 minute by varying extraction temperature at 25°C, 40°C, 50°C and 60 °C

Fig. 6: Percent DPPH assay scavenging o at 240 minute by varying extraction temperature at 25°C, 40°C, 50°C and 60 °C

From the results as shown in figure 7, it was indicated that there was a significant difference between TPC at extraction by solvent to solid ratio between 10:1 and 15:1. After extraction ratio was raised up to 20:1, the phenolic yield increased slightly with no significant distinction. This term could be explained in term of mass transfer principles that is the driving force during mass transfer within the solid was greater when a more solvent-solid ratio was used in system, resulting in a rise of the internal diffusion rate. Nevertheless, the solvent-to solid ratio did not significantly affect diffusivity when equilibrium was reached.
Fig. 7: Extraction yield at 240 minute by using solvent to solid ratio to 10:1, 15:1 and 20:1 (ml/g)

Fig. 8: % DPPH assay scavenging at 240 minutes by varying ratio of solvent to solid 10:1, 15:1 and 20:1 (ml/g)

For the results of antioxidant activity by % DPPH scavenging determination in various condition influence same as total phenol. The result shown in figure 4, figure 6 and figure 8 respectively. As the values of % DPPH shown it can be proved that antioxidant activities and phenolic contents were highly correlate. Furthermore, this trend could be explained that increasing of ethanol concentration to 70% (v/v), operation temperature to 50°C and using solvent to solid ratio of 20 : 1 (ml/g) would provide the optimum condition by resulting in high total phenolic compound and antioxidant activity.

Fig. 9: Effective diffusivity at 240 minutes by varying extraction temperature at 25°C, 40°C, 50°C and 60 °C

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From the result as shown in Fig. 9 total phenolic diffusion coefficients have increased $6.79 \times 10^{-7}$ m$^2$/s to $13.2 \times 10^{-6}$ m$^2$/s with increasing extraction temperatures from 25°C to 50°C. This trend could be described that the increase in diffusivity due to temperature may be caused by an escalation of the internal energy in molecules and an improvement their mobility, including a dwindling of the dynamic viscosity coefficient.

4. Conclusion

Results exposed batch extraction is an preliminary technique for antioxidant extraction from W.globosa with various advantages in terms of cost, time and yield. Wolffia Globosa plant is the newly noticeable source of bioactive compounds in the derivative of phenolic compounds because of their economic cost.

5. Acknowledgements

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6. References


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