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Effect of Phase Composition on Partition Behaviour of Bioactive Phenolics Transfer in Aqueous Two-Phase Extraction System

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Abstract The present study was to develop a cost-effective and eco-friendly separation and purification method of bioactive phenolics from *Phanerochaete chrysosporium* biomass using aqueous two-phase extraction system. The extraction system comprised of 80% (w/w) ethanol, 20% (w/w) di-potassium hydrogen phosphate, distilled water and solid biomass. The extraction was conducted at different phase composition range, which are 70 wt%, 50 wt% and 45wt% which paired with 27 wt%, 44 wt% and 50wt% concentrations from stock solutions of 80% (v/v) of ethanol and 40% (w/v) of K_2HPO_4, respectively. The highest partition coefficient (K) and recovery (R) of bioactive phenolics were found at 70 wt% of 80% (v/v) C_2H_5OH and 27wt% of 40% (w/v) $K_2HPO_4$90 with values of 10.45 and 99.27%, respectively.

Keywords Aqueous Two-Phase Extraction, Phenolics, *Phanerochaete chrysosporium,* Partition Behaviour

1. Introduction

Natural phenolic compounds are low molecular weight naturally occurring organic compounds which contains one or more phenolic group. They are naturally produced by plants and basidiomycete. Natural phenols also play many significant roles in human health as evident from their antifungal, antioxidant and anti-cancerous activities [1]. *Phanerochaete chrysosporium* is a filamentous basidiomycete white rot fungus that participates in the degradation process of complex woody materials. This fungus has great potential in many biotechnological applications including bioprotein production, the treatment of hazardous waste and the bioremediation of contaminated soils and biofuel production [2]. Owing to the increasing interest in new natural sources of antimicrobial compounds, this is the first study on *Phanerochaete chrysosporium* mycelia biomass as potential source of bioactive compounds.

Extraction is the most important step in the recovery and purification of bioactive compounds from plants and basidiomycetes. Phenolic compounds are usually extracted from natural sources through solid-liquid extraction using organic solvents in heat-reflux systems [3]. However, this method has suffered low efficiency, time and solvent consuming, could lead to the degradation of bioactive phenolics and decrease the bioactivity of the extracts. Aqueous two-phase extraction is recognized as an effective, versatile, easy constituent recovery and reutilization, reduced settling times, and low viscosity. [4] It will achieve high product purity as well as high yield, while maintaining the biological activity of the molecule, which has been widely applied in the separation of proteins, enzymes, antibiotics and polyphenols [5].

In the present study, the effect of phase composition on the partitioning of the bioactive phenolics from *Phanerochaete chrysosporium* mycelia biomass of the extracts were investigated in order to develop an efficient aqueous two-phase system for the extraction and purification of these compounds.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent standards, and other solvents and reagent of analytical grade were purchased from Merck Chemicals (Darmstadt, Germany).

2.2. Fungal Strain and Preparation of Inoculum

The white rot fungus *Phanerochaete chrysosporium* was obtained from the culture collection of School of Bioprocess Engineering, University Malaysia Perlis. Four square plugs (5 mm in length) of active mycelia were cut from the initial culture plate and transferred on new potato dextrose agar (PDA) plate. The plate was incubated at 30°C for 5 days. The

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inoculum was prepared by washing four PDA plates cultured with 100 ml of sterile water and their spore suspension were rubbed and poured into 250 ml Erlenmeyer flask.

2.3. Submerged Fermentation

The fermentation was done at fixed media compositions. The media was supplemented with malt extract broth to enhance the strain's growth. Fifty millilitres of sample were taken into 100-ml Erlenmeyer flasks and autoclaved at 121°C for 30 min. The sterile media was cooled to ambient temperature and inoculated with spore suspension of *Phanerochaete chrysosporium*. The fermentation was carried out at incubation time of 3 days, 4% (v/v) of inoculum size, inoculum age of 7 days, pH 6, agitation speed of 100 rpm, and fermentation temperature of 25°C. For reproducibility of results, all fermentations were carried out in triplicate.

2.4. Preparation of Mycelia Biomass Powder

After fermentation, the mycelia biomass of *Phanerochaete chrysosporium* was collected by filtration using filter paper and rinsed with distilled water. The biomass was dried in oven at 60°C for 24 h and ground into powder by using mortar and pestle. The phase system was prepared in a 15ml graduated centrifuge tubes by weighing the appropriate amount use which is 80 wt. % of ethanol, 40 wt. % of potassium salts and 0.028g of sample powder. Distilled water was added to the system to obtain final mass of 14g. The phase system was mixed evenly by gentle agitation, after that each phase system was centrifuged at 3000 g for 10minutes to induce phase separation. The phase diagram used was developed using cloud point method. The extraction was conducted at different phase composition range, which are 70 wt%, 50 wt% and 45wt% which paired with 27 wt%, 44 wt% and 50 wt% concentrations from stock solutions of 80% (v/v) of ethanol and 40% (w/v) of K_2HPO_4, respectively for 2 hours at pH 7 and temperature 25°C .

The partition coefficient (K) and the percentage of recovery, R (%) of bioactive phenolic were calculated as follows:

$$K = \frac{C_T}{C_B} \quad (1)$$

$$R_T(\%) = \frac{100 C_T C_B}{(C_T C V_B + C_B V_B} \quad (2)$$

$$R_B = \frac{100 C_B V_B}{(C_T V_T + C_B V_B} \quad (3)$$

CT and CB represent the concentrations of bioactive phenolics in the top and bottom phases, respectively. *VT* and *VB* are the volumes of the top and bottom phases, respectively.

2.5. Determination of Total Phenolic Content

The total phenolic content was determined based on the suggested method with modification [2], using the Folin-Ciocalteu reagent with gallic acid as a standard. In 15 ml test tube, 2.37 ml of distilled water, 0.03 ml of sample extract or blank and 0.15 ml of Folin-Ciocalteu reagent were added and vortexed. After 1 min, 0.45 ml of 20% saturated sodium carbonate (Na_2CO_3) was added, and then the mixture was vortexed and allowed to stand at 40°C for 30 min. The absorbance was taken at 750 nm. The total phenolic content was expressed as mg of gallic acid equivalent per liter (GAE mg/l). All measurements were measured in triplicate.

3. Results and Discussions

3.1. Partition Behaviour of Bioactive Phenolics in Aqueous Two-Phase Extraction System

The extraction of bioactive phenolics was conducted different phase composition range, which 70 wt%, 45 wt% and 25wt% which paired with 27 wt%, 44 wt% and 50 wt% concentrations from stock solutions of 80% (v/v) of ethanol and 40% (w/v) K_2HPO_4, respectively for 2 hours at pH 7 to investigate their effect on the separation of bioactive phenolics from *Phanerochaete chrysosporium* biomass using aqueous two-phase extraction system. The optimum extraction system comprised of 80% (w/w) ethanol, 40% (w/w) di-potassium hydrogen phosphate and distilled water was selected from the phase diagram of ethanol with K_2HPO_4 at 25°C shown in Figure 1.

The partitioning of bioactive phenolics in ATPS was evaluated in term of concentration of phenolics, partition coefficient (K) and percentage of recovery (R). These parameters have close correlation with the total phenolic content (TPC) extracted from crude powdered biomass in which this quantitative determination was performed via Folin-Ciocalteu (FC) assay.

From the phase diagram as shown in Figure 1, a tie-line could be drawn to allow selection of three dissimilar points at the beginning, middle and end part of the straight line which were to be used to perform ATPE on crude fungal (*P. chrysosporium*) biomass. The concentrations of ethanol and K_2HPO_4 selected from the phase diagram were 70 wt%, 50 wt% and 45wt% which paired with 27 wt%, 44 wt% and 50 wt% concentrations from stock solutions of 80% (v/v) of ethanol and 40% (w/v) of K_2HPO_4, respectively.

From the figure 2 the highest percentage of recovery of phenolics in ethanol-rich phase was determined for the first phase composition which was 99.27%, followed by the second and third with the values of 90.80% and 70.71%, respectively when ethanol concentration increased. Also, the percentage of recovery in K_2HPO_4-rich phase showed increment from 0.73% to 16% and lastly, 29% when there was higher salt concentration which indicated less effective bioactive phenolics partitioning towards the top phase. Accordingly, by referring to all results stated above, it could be concluded that the most ideal ratio of phase composition suitable for ATPS was the combination of 70 wt% C_2H_5OH and 27 wt% K_2HPO_4. The concentration of phenolics, partition coefficient and percentage of recovery in top phase declined when ethanol concentration decreased from 70 wt% to 50 wt% and lastly, 45 wt%. This in turn revealed the fact

that the higher the ethanol concentration, the higher the tendency of bioactive phenolics to partition into this phase as there was more ethanol molecules in the higher volume of top phase. Additionally, higher concentration of ethanol was capable of resulting in increasing propensity of the hydrophobic molecules in repelling water out of the phase which then led to accumulation of too much water that entered into the K_2HPO_4-rich phase [6], hence, increased quantity of target extracts that migrated preferentially towards the desired ethanol-rich phase due to similarity in polarity.

When there was an increase in K_2HPO_4 concentration from 27 wt% to 44 wt% and lastly 50 wt%, attraction of excessive water molecules especially for the last salt concentration would result in the reduction of volume of ethanol-rich phase which hindered the system to perform in an effective manner, thus reducing ability in extracting or recovering the bioactive phenolics since the phase polarity might be inappropriate for efficient hydrophobic interaction with the phenolic constituents [7]. Furthermore, the precision of results obtained could also be validated when ATPE was conducted by involving last phase composition (45 wt% C_2H_5OH and 50 wt% K_2HPO_4) in which the partitioning of solutes of interest was less desirable as indicated by the lowest K and R values. This was mainly because there was larger proportion of bottom phase compared to that of top phase, leading to an undesired difference in volume between both phases which rendered the extraction process to be ineffective [8].

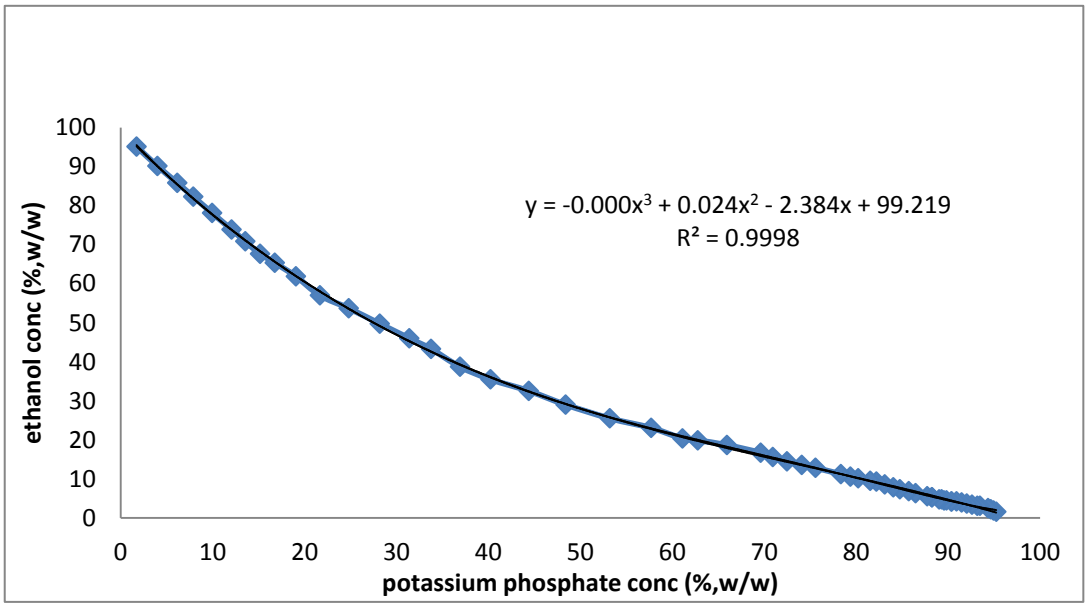

Figure 1. Phase Diagram of Ethanol + K_2HPO_4 + H_2O at 298.15 K

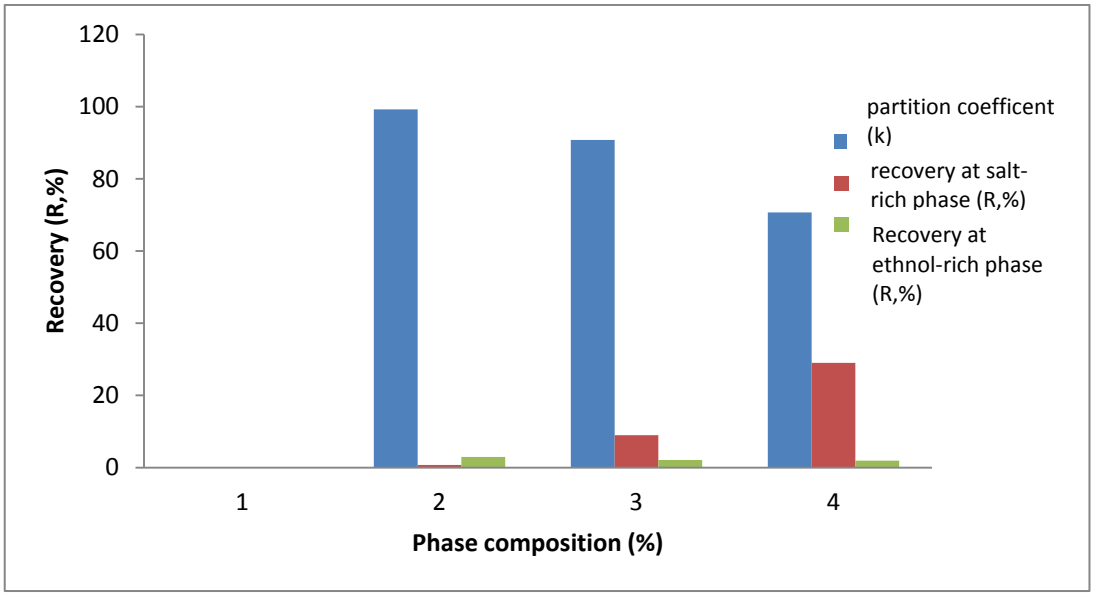

Figure 2. Effect of extraction phase composition on the partition coefficient (K) and recovery of bioactive phenolic in ethanol/K_2HPO_4 based system

4. Conclusions

This study demonstrated that the aqueous two-phase extraction system consisted of 80% (w/w) ethanol, 40% (w/w) di-potassium hydrogen phosphate and distilled water was successfully separated and purified bioactive phenolics from *Phanerochaete chrysosporium* biomass. The highest partition coefficient (K) and recovery (R) of bioactive phenolics were found at phase composition with values of 10.41 and 99.27%, respectively. For further study, the thermodynamics analysis and the identity of the phenolic compound will be investigated.

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REFERENCES

[1] M. A. Ansari, A. Anurag, Z. Fatima S. Hameed, Natural Phenolic Compounds: A Potential Antifungal Agent, Microbial pathogens and strategies for combating them: *science, technology and education* (A. Méndez-Vilas, Ed.), 2013.

[2] P. Jamal, Z. M. Idris, & M. Z. Alam, Effects of physicochemical parameters on the production of phenolic acids from palm oil mill effluent under liquid-state fermentation by *Aspergillus niger* IBS-103ZA, *Food Chemistry, 124(4), 2011,* pp. 1595-1602.

[3] L. Wang, & C. L. Weller, Recent advances in extraction of nutraceuticals from plants, *Trends in Food Science & Technology, 17*, *2006,* 300–312.

[4] M. R. Palamores, Aqueous Two Phase system, Comprehensive Biotechnology, Second Edition, 2011, Vol. 2, pp 697-713.

[5] X. Wu, L. Liang, T. Zhao, J. Zhao, F. Li, L. Yang, Aqueous two-phase extraction, identification and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.), *Food Chemistry, 129, 2011,* pp. 443-453.

[6] Liu, L., Dong, Y. S. & Xiu, Z. L. (2010) Three-liquid-phase extraction of diosgenin and steroidal saponins from fermentation of *Dioscorea zingibernsis*. C. H. Wright. *Process Biochemistry*, *45*(5), 752-756.

[7] Zhang, D. Y., Zu, Y. G., Fu, Y. J., Wang, W., Zhang, L., Luo, M., Duan, M. H. (2013). Aqueous two-phase extraction and enrichment of two main flavonoids from pigeon pea roots and the antioxidant activity. *Separation and Purification Technology, 102*, 26-33.

[8] Ma, F. Y., Gu, C. B., Li, C. Y., Luo, M., Wang, W., Zu, Y. G., Fu, J. Y. (2013). Microwave-assisted aqueous two-phase extraction of isoflavonoids from *Dalbergia odorifera* T. Chen leaves. *Separation and Purification Technology, 115*, 136-144.