

## Optimisation of Extraction of *Phaleria Macrocarpa* Leaves

Nor Fariza<sup>1\*</sup>, Luqman Chuah A<sup>1,2</sup>, Pin KY<sup>3</sup>, Dayang Radiah AB<sup>1</sup>, Umi Kalsom Y<sup>4</sup> and Adawiah I<sup>1</sup>

<sup>1</sup>Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, Malaysia

<sup>2</sup>Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, Malaysia

<sup>3</sup>Forest Research Institute of Malaysia (FRIM), Malaysia

<sup>4</sup>Department of Biology, Faculty of Science, Universiti Putra Malaysia, Malaysia

### Abstract

*Phaleria macrocarpa* (Scheff.) Boerl known as mahkota dewa has been used traditionally to treat cancer, impotency, diabetes mellitus, heart disease, blood pressure and various skin diseases by Indonesians. Through recent study, this plant has been proven to treat cancer. Phalerin is one of the major compounds in this plant which is believed to contribute to cure cancer cell. Thus, this study aims to investigate the effect of solid-to-solvent ratio, particle sizes and temperature on the yield of extract and concentration of phalerin. The result obtained from this experiment enabled one to optimize and develop the extraction kinetics model. The solid liquid extraction is used to extract the compounds from the plants. The process is carried out by using water as solvents and using leaves at various particle sizes, solid-to-solvent ratio of 1:10 to 1:50 (g/ml), temperature of 40 to 80°C and last up to 5 hours. The crude extract was analyzed to determine the yield and HPLC analysis was used to determine the concentration of phalerin present. From the study, the optimum parameters for the extraction phalerin from *Phaleria macrocarpa* leaves are at 70°C using 1:20 (g/ml) solid-to-solvent ratio and particle size of <250 µm for 120 minutes.

**Keywords:** *Phaleria macrocarpa*; Phalerin; Solid liquid extraction; Optimisation

### Introduction

*Phaleria macrocarpa* (Scheff) Boerl is a medicinal plant originally from Papua, Indonesia. This plant also known as God's crown, has been used by the traditional folk from Indonesia to treat cancer, diabetes, common cold, viral infections, allergy problem, asthma, cardiovascular, high blood pressure, acne and insect bites [1]. Moreover, it has exhibited potential medicine to cure diseases such as cancer, impotency, diabetes mellitus, heart disease, blood pressure and various skin diseases. The study on *Phaleria macrocarpa* has been widely done mainly to investigate the potential usage on parts of the plant including leaves, fruits, bark and seed.

Phalerin or 4,5-dihydroxy,4'-methoxybenzo-phenone-3-O-β-D-glucoside was initially identified by Wahyuningsih et al. [2]. It was found in leaves and fruit of *Phaleria macrocarpa*. This particular compound was cytotoxic to myeloma cell line (NS-1) through *in vitro* study by having IC<sub>50</sub> of 83 µg/ml or 1.9×10<sup>-1</sup> mM and also cytotoxic towards EVSA-T (breast cancer with estrogen negative receptor) with IC<sub>50</sub> 1.37×10<sup>-1</sup> mM. The methanol extract of *Phaleria macrocarpa* consist of phalerin showed mild anti-inflammatory effect [3]. Moreover, phalerin of 500 µg/mL concentration can significantly increase (p<0.05) the p53 protein (protein tumor suppressor) expression compared with control [4].

Since phalerin is a major component in this plant and possess bioactivity effect, an effort to optimize the production of phalerin through extraction needs to be studied. This study focus on the optimization of extraction process of *Phaleria macrocarpa* leaves extract as well as the major component, phalerin. The aims of the study are to investigate the effect of extraction parameters on the concentration of phalerin in the extract and the kinetics study on the extract.

### Materials and Methods

#### Materials

The *Phaleria macrocarpa* leaves were obtained from Yaacob Berkat

Enterprise. The leaves was initially dried, grind and sieve using sieve shaker into particle size in range of <250, 250-500, 500-1000 and >1000 µm. The phalerin was obtained through extraction process by separation and fractionation of the extract components [5].

#### Optimization process of the phalerin in *Phaleria macrocarpa* extract

Extraction of phalerin from *Phaleria macrocarpa* leaves was done using solid liquid extraction method. Water was used as solvent due to safety, low cost and environmental friendly. Moreover, the targeted compound, phalerin, was soluble in water. The process was done using water bath extraction where 5 g of sample was immersed in 200 ml of solvents for 5 hours. Then, the mixture was filtered using Whatman No. 1 filter paper. The crude extracts of *Phaleria macrocarpa* leaves was dried using freeze dryer (Model FD8, Heto, USA). Later, phalerin concentration in the extract was determined using High Performance Liquid Chromatogram (HPLC) analysis.

The study on the effect of solid-to-solvent ratio was conducted as above method. The solid-to-solvent ratio was varied by 1:10, 1:20, 1:30, 1:40 and 1:50 (g/ml). Then, extract was filtrated and freeze dried to remove the solvents.

The study on the effect of particle size was conducted as above method. The particle size of the sample used were in range of <250,250-

**\*Corresponding author:** Nor Fariza Ismail, Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Tel: 019-9821412; E-mail: [ezza\\_nfi14@hotmail.com](mailto:ezza_nfi14@hotmail.com)

**Received** December 05, 2013; **Accepted** January 22, 2014; **Published** January 26, 2014

**Citation:** Fariza N, Luqman Chuah A, Pin KY, Dayang Radiah AB, Umi Kalsom Y, et al. (2014) Optimisation of Extraction of *Phaleria Macrocarpa* Leaves. Med Aromat Plants 3: 149. doi: [10.4172/2167-0412.1000149](https://doi.org/10.4172/2167-0412.1000149)

**Copyright:** © 2014 Fariza N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

500,500-1000 and >1000  $\mu\text{m}$ . Then, the extract was filtered and freeze dried. The HPLC was used to determine the phalerin present.

The study on the effect of temperature was performed as above method with operating temperature varied at 40,50, 60,70 and 80°C. Then, extract was filtrated, freeze dried analyzed using HPLC.

### HPLC analysis to determine phalerin concentration in the extract

The concentration of phalerin present in the extract was determined through HPLC analysis. An HPLC system (Waters Delta 600 systems) equipped with a photodiode array detector (Water 996), autosampler (Waters 717 plus) and Empower software was used. The separation was conducted using a Phenomenex Luna C18 column using 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phase running in gradient mode. An injection volume of 30  $\mu\text{L}$  with a 1 mL/min of flow rate was used. Spectral information over the wavelength range of 200 to 400 nm was collected.

The phalerin concentration in the extract was determined by comparing the peak area of the extract and the standard at the same retention time. The area under each peak is proportional to the concentration of that component in the standard phalerin.

### Kinetic study

The extraction for kinetics study was performed using an extractor which was a 60-liter-jacketed vessel equipped with a top-mounting motor for agitation and internal heater for heating. The agitation speed controller and temperature controller were used to regulate the agitator speed and heating temperature respectively. The agitation speed was controlled at 30 rpm for the experiments.

The extraction kinetics study was done using water as solvent at optimum parameter which were 1:20 (g/ml) solid-to-solvent ratio and particle size in range of <250  $\mu\text{m}$ . The extraction was carried out at temperature of 40, 60 and 70°C for 4 hours. The extract was sampled for every 5 minutes in the first 20 minutes, every 10 minutes in the next 40 minutes, every 30 minutes for the next 2 hours for total of 4 hours operating duration. The extracts were filtered using Whatman No. 1 filter paper assisted with vacuum to remove residue particles. The filtrate was freeze dried to remove the solvent and yield was determined.

## Results and Discussions

### Effect of solid-to-solvent ratio

Solid-to-solvent ratio effect on the phalerin concentration is shown in Figure 1. From the study, the concentration of phalerin increases as the solid-to-solvent ratio change from 1:10 to 1:50 (g/ml). Pinelo et al. [6] reported that the lower the solid-to-solvent ratio, the higher the amount of extracted solid obtained. However, beyond 1:20 (g/ml) solid-to-solvent ratio, the concentration of phalerin was no longer increased to a significant amount which implied that the system was already at equilibrium and the solid content was the limiting factor. Thus, the optimum solid-to-solvent ratio was 1:20 (g/ml) with the concentration of phalerin of  $297.28 \pm 5.41$  ppm.

### Effect of particle size

Figure 2 shows the effect particle sizes on the phalerin concentration. The smallest particle size favored the mass transfer of the solid content into the solution. From a diffusion point of view, smaller particle size provides a better access of solvent into the pores of the leaves (not only

the meso but to some of the micro pores) [7]. This was due to large surface area that provide contact surface which created concentration gradient for the mass transfer to occur. The study on the effect of particle size showed that the optimum particle size was <250  $\mu\text{m}$ .

### Effect of temperature

Figure 3 shows the effect of temperature on the phalerin concentration. The concentration of phalerin increases as temperature increases. The highest concentration of phalerin was found at temperature of 70°C. However, beyond 70°C, the phalerin concentration was decreasing. From this study, high temperature is favor to improve the efficiency of extraction as it enhances the diffusion rate and solubility of phytochemicals in the solvents. However, too high temperature may degrade the phytochemicals compound [8]. Moreover, phytochemical compound may also losses due to evaporation or reaction with other compound and thus affect its bioactivity.

### Extraction kinetics

From Figure 4, the yield of extract was increasing with time and maintain almost constant after 120 minutes of extraction. Despite the operation temperature, the reaction reached constant at almost the

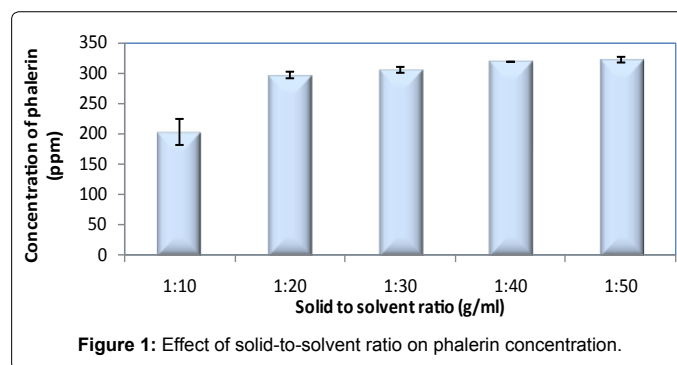


Figure 1: Effect of solid-to-solvent ratio on phalerin concentration.

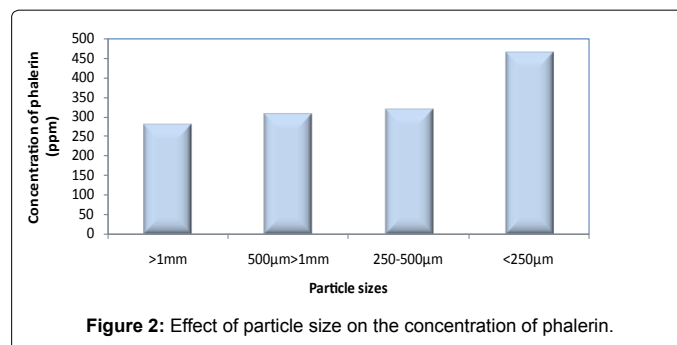


Figure 2: Effect of particle size on the concentration of phalerin.

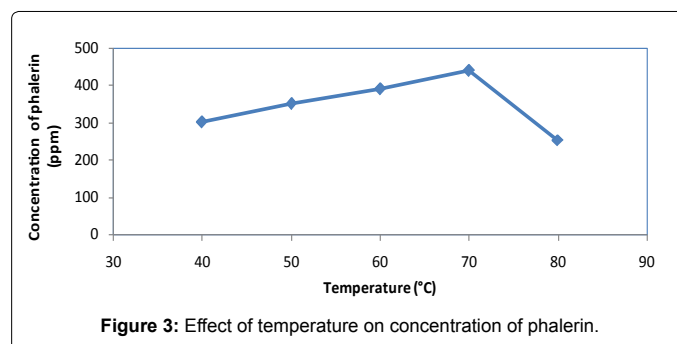


Figure 3: Effect of temperature on concentration of phalerin.

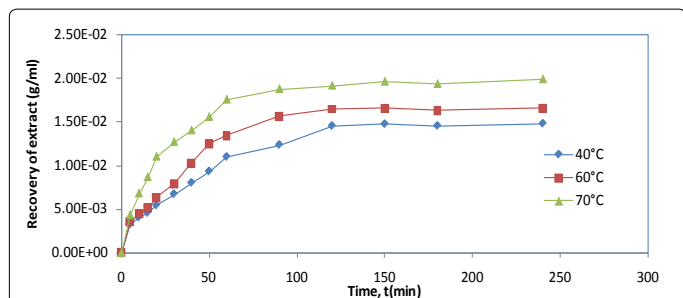


Figure 4: The yield of extract for 240 min at temperature of 40, 60 and 70°C.

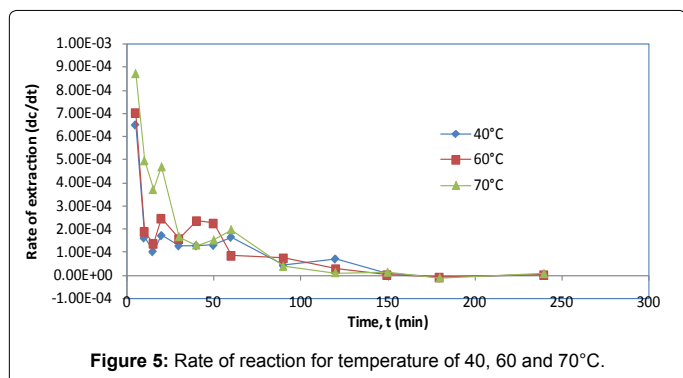


Figure 5: Rate of reaction for temperature of 40, 60 and 70°C.

same duration. However, temperature plays a role in increasing the equilibrium state of the extraction reaction as phalerin concentration is extracted the most using 70°C followed by 60°C and 40°C. This study implies that temperature was the limiting factor and the extraction process reached the equilibrium state at about 120 minutes. Thus, for the extraction process which aims for phalerin, the process should be conducted at maximum temperature of 70°C to avoid degradation of compound and last for no longer than 120 min for energy savings.

Figure 5 shows a high reaction rate at the very beginning of the extraction process. Initially, the soluble components are dissolved quickly into the solution and form a very large concentration gradient in the solid. Later, extraction rate tends to fall due to reverse flow of solvent when the leaves are swollen, hindered the transfer of solute to the surface of solid. Then, the diffusion stage takes over when the leaves are already swollen and the solute are not easily accessible. At this stage the extraction rate is slower and diffusion mechanism takes place. This study shows similar phenomena for extraction process done by Linares et al. [9].

## Conclusion

From this study, the effect of the operating parameter; solid-to-solvent ratio, particle size and temperature were successfully studied. Phalerin is a compound that dissolved in water and can be found in *Phaleria macrocarpa* leaves. When operating the extraction at smaller solid-to-solvent ratio, large amount of phalerin can be extracted.

Moreover, the smaller the particle size of sample used, the higher concentration of phalerin can be extracted. High temperature, on the other hand, promotes high amount of extracted compound.

In conclusion, the phalerin concentration using water as solvent by solid liquid extraction method was optimum when operating at 1:20 (g/ml) solid-to-solvent ratio, particle size of <250  $\mu\text{m}$  and temperature of 70°C. Through kinetics study, the extraction process reached equilibrium at 120 minutes. The maximum amount of extract was obtained using the operating temperature of 70°C.

## Acknowledgement

The authors would like to thank Mohd Farhan Abdul Razak, Nurhazwani Mohd Hirmizi, Masitah Taini and all staff of Medicinal Plant Program of Forest Research Institute Malaysia (FRIM) for their support and technical assistance. The authors would acknowledge the Malaysian Higher Education for the financial support of this study.

## References

1. Harmanto N (2003) Conquering Disease in Unison with Mahkota Dewa, *Phaleria macrocarpa*. 1<sup>st</sup> (edn) Mahkotadewa PT, Indonesia, Jakarta, 14.
2. Wahyuningsih MST, Mubarika S, Gandjar IG, Hamann MT, Rao KV, et al. (2005) Phalerin, a new benzophenone glucoside isolated from the methanolic extract of Mahkota Dewa [*Phaleria macrocarpa* (Scheff.) Boerl]. leaves 16: 51-57.
3. Nor Fariza I, Fadzureena J, Zunoliza A, Luqman Chuah A, Pin KY, et al. (2012) Anti-inflammatory Activity of the Major Compound from Methanol Extract of *Phaleria macrocarpa* leaves. J Appl Sci 12: 1195-1198.
4. Wahyuningsih MST, Mubarika S, Wahyuono S (2008) Effect of phalerin isolated from *Phaleria macrocarpa* (Scheff) Boerl. leaves on EVSA-T p53 protein expression in vitro. J Trad Med 13: 83-89.
5. Ismail NF, Ismail A, Chuah TG, Abdullah Z, Li AR, et al. (2011) Extraction, Separation and Identification of Phalerin from *Phaleria macrocarpa* (Scheff.) Boerl. In Proceeding of the International Conference on Chemical Innovation (ICCI 2011), Terengganu, Malaysia, 111-114.
6. Pinelo M, Rubilar M, Jerez M, Sineiro J, Núñez MJ (2005) Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. J Agric Food Chem 53: 2111-2117.
7. Simeonov E, Koleva V (2012) Solid-liquid Extraction of Tannins from *Geranium Sanguineum* L -Experimental Kinetics and Modelling, Chem Biochem Eng Q 26: 249-255.
8. Pin KY, Luqman Chuah A, Abdull Rashih A, Rasadah MA, Law CL, et al. (2011) Solid-liquid extraction of betel leaves (*Piper Betle* L.). J Food Proc Eng 34: 549-565.
9. Linares AR, Hase SL, Vergara ML, Resnik SL (2010) Modeling yerba mate aqueous extraction kinetics: Influence of temperature. J Food Eng 97: 471-477.