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Short communication

Optimization of mangiferin extrated from *Phaleria macrocarpa* fruits using response surface methodology

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ABSTRACT

Phaleria macrocarpa locally known as Mahkota Dewa is one of the important medicinal plants that originates from Papua Island, Indonesia and grows in tropical areas. Mangiferin is one of the major bioactive compounds of *Phaleria macrocarpa*. The effect of extraction time (4–6 h) and extraction temperature (90–110 °C) on the mangiferin yield were investigated using Face Centered Central Composite Design (FCCCD) with five centre points under Response Surface Methodology (RSM). The presence of mangiferin in the extract was confirmed using HPLC-DAD and the functional groups were identified through FTIR analyses. A second order polynomial model was employed in predicting the response. The regression analysis showed that more than 98% of the variation was explained by the models with the optimum of 38.7 mg/g mangiferin yield at 105 °C and 6 h extraction time. The experimental values show good accuracy with those predicted (1.1% deviation), thus indicating the suitability of the model employed and the success of FCCCD in optimizing the extraction conditions.

1. Introduction

Phaleria macrocarpa is one of the medicinal plants that grows in Malaysia and Indonesia. Empirically, *Phaleria macrocarpa* is utilized for medical treatment. There are four major parts of this plant that are mostly enriched in medicine, they are the stems; the leaves; egg shell of the seeds; and the fruits. The stems had been used in the treatment of bone cancer (Ashikin 2014; Fariza et al., 2012); the leaves had been used for impotence, blood diseases, allergies, diabetes and tumor treatments (Alara et al., 2016; Ali et al., 2012; Govindappa, 2015; Parhizkar et al., 2015); the egg shell of seeds were used for breast cancer, cervix cancer, lung diseases, liver and heart diseases treatments (Alara et al., 2016; Fariza et al., 2012); and the fruits consisting of alkaloid, saponin, flavonoid and polyphenol had been used as antioxidants (Andrean et al., 2014; Faried et al., 2016).

Mangiferin which is one of the major bioactive compounds of *Phaleria macrocarpa* had been researched to decrease the blood glucose level by increasing the level of insulin as well as the activity of enzymes pathways involved in glucose metabolism (Sellamuthu et al., 2009). Mangiferin had also been reported to reduce the blood glucose level through its inhibitory action on glucose absorption from the intestine of rats thus, proving its pancreatic and extra-pancreatic targets of action. It had been reported to protect the D (+) galactosamine mediated oxidatively stressed kidney tissue in rats (Pan et al., 2016). Mangiferin had been reported to be present in *Phaleria macrocarpa* fruits (Kim et al., 2010). Response Surface Methodology (RSM) is an efficient tool used for optimizing a multivariable process (Liu et al., 2006). It was further reported that Face Centered Central Composite Design (FCCCD) showed the correlation between extraction factors and their responses at the different optimum level (Liu et al., 2006).

Due to the presence of mangiferin in *Phaleria macrocarpa* fruits, and their importance in the treatment of different ailments, an effort to optimize the mangiferin production through extraction needs to be studied (Ali et al., 2012; Govindappa 2015; Alara et al., 2016; Kim et al., 2010). The aim of this research is to study the effects of extraction time and temperature on the yields of mangiferin.

2. Materials and methods

2.1. Materials

Phaleria macrocarpa dried fruits peels supplied by Ethno Resources Sdn Bhd, Selangor was used. The fruit peels were ground using a grinder (WSM grinder, West Salem Machinery, Oregon) and sieved using 200 μm mesh size. Methanol, acetonitrile, acetic

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Table 1
Independent variables and their levels used for face centered central composite design (FCCCD).

Independent variables	Factors levels		
	−1	0	+1
x_1:Temperature	90	100	110
x_2: Extraction time	4	5	6

acid, and mangiferin standard used for HPLC-DAD analysis obtained from Merck Sdn Bhd Selangor were of analytical grade. Distilled water from the laboratory was used in this experiment as the extracting solvent. All the materials were used without further purification.

2.2. Design of experiment

Response surface methodology was used to optimize the screened variables (temperature and extraction time). These were carried out by conducting the statistically designed experiments, evaluating the coefficients in the mathematical models, predicting the responses, and validating the adequacy of the model. The optimization for extraction yields of mangiferin from *Phaleria macrocarpa* fruits was done using Face Centered Central Composite Design (FCCCD) under the Response Surface Methodology (RSM) available in the Design Expert software (Version 6.0.8, Stat-Ease Inc., Minneapolis, USA). Through FCCCD, a set of 13 experimental runs with five replicated centre points (Run 9, 10, 11, 12 and 13) were designed. Three different levels (−1, 0 and +1) of independent variables were studied. The independent variables and their levels used in this study are shown Table 1. Analysis of variance (ANOVA) was used to analyze the experimental data. The adequacy of the developed response surface model was determined by evaluating the lack of fit and coefficient of determination (R^2).

2.3. Extraction of mangiferin from Phaleria macrocarpa fruits

The extraction processes were carried out using a built subcritical water extraction system. The solvent used was distilled water to ensure cost effectiveness of the extraction process. For the first run, *Phaleria macrocarpa* sample of 25 g was mixed with 1 L of distilled water, the mixture was then poured into the extraction reactor tank of the subcritical water system. The temperature of the extraction medium was adjusted between 90 to 110 °C and time 4–6 h, according to the experimental design matrix design using FCCCD with five centre points. The water level of the cooling vessel was checked and the cooling temperature was regulated to 27 °C for 30 min after the extraction process. Batch extraction was then carried out for an intended period of extraction time. After the extraction process, the mixture was removed and decanted before centrifuged at 5000 rpm for 30 min. The extracts were then stored in 4 °C refrigerator before HPLC-DAD and FTIR analyses were carried out.

2.4. HPLC-DAD and FTIR analyses

Mangiferin was quantitatively determined with the use of High Performance Liquid Chromatography (HPLC-DAD). 10 ml amount of extracts was collected using a 20 ml syringe and were dissolved in 20 ml of methanol. The methanolic extracts were analyzed with HPLC-DAD. The HPLC system of Agilent 1100 LC series system with Quaternary Pump and Gand G1315B DAD (USA) was used. The machine comprised of an 1100 Solvent Tray, 1100 Degasser, 1100 Quaternary Pump, 1100 Autosampler and 1100 DAD with Standard Flow Cell. A column (Agilent Zorbax C18 5 mm, 25 cm, 0.46 mm, USA), and built-in software, Autochro 2000 (USA). The wavelength of 280 nm was used for the chromatographic separations (Kim et al., 2010). The mobile phase was prepared according to Kim et al. (2010) method with some modifications, acetonitrile-water solution (98%) and acetic acid-tertrafurane solution (2%) at 1.0 ml/min flow rate. The amount of sample injection was set at 20 ml and HPLC-DAD was calibrated with standard solutions of mangiferin of different concentrations. The standard solutions of mangiferin were first prepared by dissolving 0.5 mg of mangiferin in 1 ml methanol. These mixtures were diluted to obtain solutions of different concentrations in the range of 0–500 ppm, which was used in constructing the calibration curve of mangiferin.1 mg of the *Phaleria macrocarpa* extract was measured using potassium-bromide (KBr) pellet method in FTIR spectrometer (Bruker-Alpha). IR data of isolated compound was compared with the reference standard of mangiferin.

Table 2
Results of the extraction of mangiferin using face centered central composite design (FCCCD).

Standard Order	Independent variables		Dependent variable
	Temperature, x_1 (°C)	Extraction time, x_2 (h)	Yield (mg/g)
1	90	4	26.20
2	110	4	34.36
3	90	6	28.76
4	110	6	37.57
5	90	5	27.83
6	110	5	36.18
7	100	4	35.25
8	100	6	38.12
9	100	5	35.87
10	100	5	37.72
11	100	5	35.85
12	100	5	35.80
13	100	5	35.88

2.5. Validation of model

The statistical model was validated with respect to the two variables within the design space. Experiments predicted by the point prediction feature of the of the Design Expert software were conducted. The results obtained were compared with the predicted values.

3. Results and discussions

3.1. HPLC-DAD and FTIR results

"Fig. 1" showed the HPLC-DAD chromatographic profile for the mangiferin standard and mangiferin extracted from *Phaleria macrocarpa* fruits at optimum condition. The retention time of 2.819 min obtained from the extract agreed well with the mangiferin standard verifying the presence of mangiferin in *Phaleria macrocarpa* fruits extract.

FTIR analysis had been reported to be an effective tool for the characterization and identification of functional groups (Eberhardt et al., 2007). The FTIR spectrum of mangiferin standard and mangiferin extracted from *Phaleria macrocarpa* fruits at the optimum condition are showed in "Fig. 2". Six functional groups were identified by the FTIR spectrum analysis results: peak at 1,417 cm^{-1} showed the presence of C—O bond, peak at 1,450 cm^{-1} reflected the presence of CH—CH bond, peak at 1,655 cm^{-1} indicated the presence C—O bond, peak at 2,942 cm^{-1} showed the presence of C—H anti-symmetric stretching, and peaks at 3,316 cm^{-1} showed the presence of secondary OH—bond. The peak at 1,023 cm^{-1} showed the presence of C—C anti-symmetric stretching in the mangiferin structure. Comparing the FTIR analysis of mangiferin extracted from mangiferin standard and *Phaleria macrocarpa* fruits, it showed

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