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Microwave Assisted Extraction of *Cyanotis beddomei*

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Abstract

Cyanotis beddomei is being extracted via Microwave-Assisted Extraction (MAE) method. The chosen variables for the extraction were methanol proportion, extraction time, extraction temperature and ratio of liquid to solid. The optimum extraction conditions were methanol of 70% (v/v), extraction time of 30 min, extraction temperature of 70°C and liquid to solid ratio of 20 mL/g. Under these conditions, 13.31 mg/g total plant extracts were obtained by Microwave Assisted Extraction (MAE). The results showed that all the chosen variables for the MAE were significant on the yield of crude extract while the most important variable was the extraction time and solid to liquid ratio.

Keywords: *Cyanotis beddomei*; Microwave-assisted extraction; Crude extract

Introduction

According to a research, the number of diabetes cases will gradually rise and by the year 2030, 439 million adults are expected to suffer from diabetes. This staggering number has caused an increase in research on diabetes as well [1].

Due to this issue getting more serious by the day, many herbal plants with reported hypoglycemic activity has received attention for researcher [2]. Medicinal plants have long been used in virtually all cultures as a source of medicine. It has been estimated that about 80-85% of population depends on traditional medicine for their primary health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles [3].

Cyanotis beddomei is in the *Commelinaceae*, a family which has accommodates several other houseplants (*Tradescantia pallida, Tradescantia sillamontana, Tradescantia spathacea* and *Tradescantia zebrina*, among others) and outdoor plants, plus a few weeds (*Commelina communis* being the main one). It is native in Africa, southern Asia, and northern Australia. Commonly known as the "Teddy-bear Plant". *C. beddomei* have flowers that are not long lasting especially on dark environment.

Cyanotis beddomei is a common houseplant that has spreading, or trailing stems combined with unique green leaves with reddish-brown underneath. Due to the nature of the plant, it is common to find this houseplant in hanging pots which allows the long tendril like stems to grow efficiently. With enough sunlight and water, this plant is capable of rapid growth and can be classified as an invasive species.

This plant is traditionally believed to aid in recovery of kidney related problems as well as improving blood circulation and urine discharge. The leaves of these plants are boiled together with red dates and taken as a supplementary drink to help improve kidney health. This medicinal effect may arise from the antioxidants and other secondary metabolites that is believed to be present in this plant. However, proper reports on the anti-diabetic and antioxidant properties of these plants have remained scarce so far.

This study aims to optimize the extraction of the bioactive compounds found in *Cyanotis beddomei* using microwave assisted extraction method. The effects of methanol proportion, temperature of extraction, solid to liquid ratio and extraction time on total plant crude extract yield were studied and optimal extraction conditions were found using response surface methodology by measuring the yield of total plant crude extract.

Materials and Methods

Materials and reagent

The plant samples were obtained from a plant nursery named N&T Orchid located in Merbok, Kedah. The chemicals and reagents used throughout the experiment were obtained from the laboratories of Faculty of Applied Sciences, Faculty of Pharmacy and Faculty of Medicine. The chemicals and reagents used are methanol (70%, 80% and 90%).

Equipment and apparatus

The microwave used in MAE is 800 W microwave of Sanyo brand. A Soxhlet extractor was used in plant sample extraction for qualitative phytochemical analysis. The spectrophotometer used in TCA is PRIM 2. The oven used for drying is MMM drying oven. The rotary evaporator used for drying of solution obtained from Soxhlet Extractor is EYELA rotary evaporator. The orbital shaker used is INNOVA 40.

Optimization of the extraction procedure

Figure 1 illustrates the overall process flow of extraction of bioactive compound in *C. beddomei* plant. There are several parameters that affect the MAE efficacy, such as the methanol proportion, extraction time, extraction temperature and liquid to solid ratio. From the preliminary test runs, appropriate ranges of methanol proportion, extraction time, extraction temperature and liquid to solid ratio were chosen. The independent variables chosen were methanol proportion (X1), extraction time (X2), extraction temperature (X3) and liquid to

solid ratio (X4). Three level of settings were adopted to optimize the MAE. The three levels of settings are coded as -1, 0 and +1 (Table 1). A total of 27 different experimental designs (Table 2) were performed in random order and all experimental results were expressed as a mean of three parallel measurements [4]. Each conical flask was filled with 1 g of *C. beddomei* powdered sample based on number of empty slots in the orbital shaker. The conical flasks were then filled with methanol of varying proportion from the experimental designs and labelled accordingly. The conical flasks were placed on the orbital shaker which is pre-heated to designated temperature of 65°C. The orbital shaker was started, and the conical flasks were collected based on their time of extraction. The plant extract was sucked out using a sterile dropper into a filter paper placed on a filter funnel. Using a micropipette, 1 ml of the plant extract was poured onto a glass petri dish. The weight of the petri dish was deducted beforehand to allow only the weight of the methanol and plant extract to be accounted later [5]. The weight of 1 ml of plant extract was recorded. The petri dish was then placed in a microwave oven for drying at medium temperature for 2 mins. The petri dish was retrieved and placed into a desiccator for cooling [6]. The new weight which corresponds to the plant extract minus the methanol was recorded. The steps were repeated for other plant extracts in the remaining conical flasks. The experiment was then repeated for the other to remaining temperatures [7].

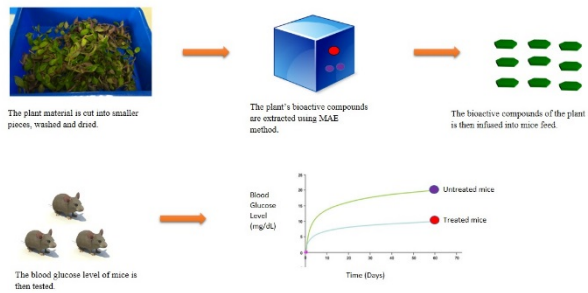

Figure 1: Overall process flow of extraction of bioactive compound in *C. beddomei* plant for anti-diabetic properties.

Independent Variable	Settings		
	-1	0	+1
Methanol Proportion (%)	70	80	90
Extraction Time (Mins)	15	30	45
Extraction temperature (°C)	65	70	75
Ratio of liquid to solid (ml/g)	20	30	40

Table 1: Code and settings for chosen parameters.

Methanol	Temperature	Time	L/S ratio	Yield of total flavonoids (Y4)	σ^2
90	70	15	30	4.2833	2.65
80	65	30	40	5.45	2.21
90	65	30	30	6.7667	0.75
80	75	15	30	6.9833	0.2
80	70	30	30	7.4	2.09
80	75	30	40	6.9667	3.94
70	70	30	20	13.3167	1.15
90	70	30	20	8.2833	1.57
80	70	45	20	8.5833	3.98
90	70	30	40	5.3667	2.15
80	70	15	40	4.8333	3.67
80	65	30	20	10.4333	0.42
80	70	15	20	6.6833	3.09
70	70	45	30	5.9	3.51
80	75	30	20	10.85	1.92
80	65	45	30	6.0667	2.55
80	75	45	30	5.8833	2.18
90	75	30	30	5.85	3.15
80	70	30	30	6.2	1.41
80	70	45	40	4.5667	2.65
70	70	30	40	5.05	2
70	75	30	30	7.4833	2.41
70	70	15	30	5.1833	3.14
90	70	45	30	5.3	2.3
70	65	30	30	8.6	0.82
80	65	15	30	5.3333	3.7
80	70	30	30	6.7	1.3

Table 2: Experimental design with chosen settings.

Results and Discussion

The effects of the chosen variables on the optimized extraction of *Cyanotis beddomei* using Microwave-Assisted Extraction (MAE) method is shown in Table 1 in the form of yield. The denotation Y4 represents the average of all the experimental runs while σ^2 represents the standard deviation of the runs [8].

Figure 2A shows the 3 different methanol concentrations used (70%, 80% and 90%) and their best corresponding yield. The best yield is obtained when 70% methanol is used, and the yield decreases as the methanol concentration increases. This is probably due to the change in the total polarity in the solvent used contributed by the increase in methanol concentration and the higher concentrations of methanol may prevent the effective dissolution of the sample's bioactive compounds [9,10]. Methanol is a good organic solvent but not as polar as water. As the concentration of the methanol increases, some highly polar compounds will not completely be extracted. Thus, a combination of 70% methanol and 30% water is the best suited solvent to extract both polar and non-polar compounds. As the concentration

of the methanol increases, some highly polar compounds will not completely be extracted [11].

Figure 2B shows the three different solid to liquid ratio (l/s) used which are 20 mg/ml, 30 mg/ml and 40 mg/ml. The yield is the highest when 20 mg/ml is used, and the yield is shown to decrease as the solid to liquid ratio increases. This shows that 20 mg/ml is the optimal ratio of solid to liquid for the extraction of this plant [12].

In Figure 2C, the temperatures used in MAE are shown. The chosen temperatures are 65°C, 70°C and 75°C. The yield is shown to be the highest in 70°C. This is probably due to shorter exposure to heat is not sufficient for complete extraction of bioactive compounds while prolonged exposure to heat may denature the compounds thus resulting in lower yield [13].

Figure 2D indicates the time of extraction that are chosen for this MAE which are 15 mins, 30 mins and 45 mins. The highest yield is observed at 30 mins and the yield of bioactive compounds at 15 mins and 45 mins are significantly lower. This shows that 15 mins is not enough for the complete extraction of *Cyanotis beddomei* while 45 mins results in extended heating and this may have adverse impact on the yield [14].

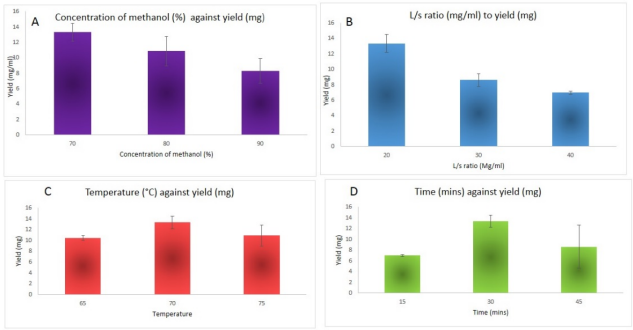

Figure 2: Effect of methanol proportion, extraction time, extraction temperature and ratio of liquid to solid on the yield of total plant extract.

The actual extraction yield of total plant extract was 13.31 mg/g under the optimization conditions at ethanol proportion of 70%, extraction time of 30 mins, extraction temperature of 70°C and ratio of liquid to solid of 20 mL/g. Compared with the maceration extraction technology, the optimized MAE can save time and energy and provide higher extraction yield of total plant extracts [15].

Statistical analysis and model fitting

Statistical analysis and model fitting are adequate to represent the relationship between the response and independent variables.

The experimental data obtained from a 27-runs-experiment are provided in Table 2. The Analysis of Variance (ANOVA) of total flavonoids yield is presented in Table 3. The results from Figure 3 define the polynomial model for the flavonoids yield as regressed in the equation below in terms of the coded factors:

Y (Yield of total flavonoids)=+6.77X1+0.11X2+0.26X3–2.17X4+0.07X1X2+0.10X2X3+1.33X1X4-0.44X2X3+0.27X2X4–0.54X3X4-0.05X1-2+0.60X2-2–1.53X3-2+1.08X4-2

With R-2=92.17%

Table 3 indicates that the ANOVA response surface quadratic regression model is highly significant ($p<0.01$) with a F value of 10.09, with the Lack-of-Fit statistic ($p=0.344$) which was used to test the adequacy of the model is not significant. There was no abnormality present from the diagnosis of residuals (Figure 3). Thus, it may be concluded that the model was statistically sound and adequate to represent the relationship between the response and independent variables.

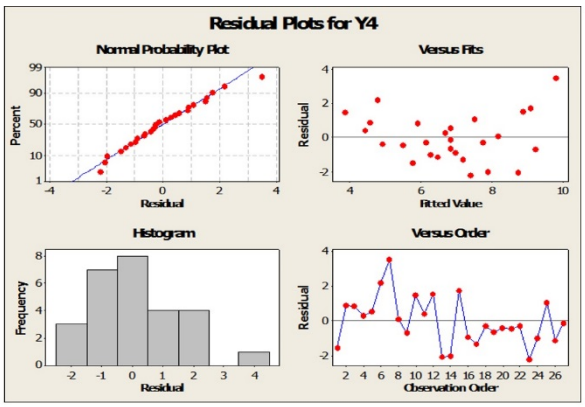

Figure 3: Residual Plots for Total Flavonoid Extract (Y4).

The linear effects of each of the independent variables within the experimental range may be deduced in Figure 4. The total flavonoids yield was affected, with a significant ratio of liquid to solid (X4) ($p<0.000$), followed by methanol (X1) ($P<0.007$). The factors time, and temperature did not show a significant effect in terms of the F value. The quadratic function was significant at ($F=10.28$) ($P<0.001$), also for Time ($F=16.59$) ($p<0.002$) which consisted of the duration of the period of extraction and the Liquid-to-solid ratio ($F=8.28$) ($p<0.014$). There was also a significant effect of the interaction of methanol to the Liquid-to-solid ratio ($F=9.50$) ($p<0.010$)

The effects of the independent variables and their interaction on the yield of flavonoids may be seen on the three dimensional response surface curves and contour plots provided in Figures 4A-4D. The plots were generated by keeping two of the variables constant, which indicated the changes in the total flavonoid yield under different conditions of the extraction protocols. The effect of methanol proportion and temperature range on the extraction of flavonoids is shown in Figures 4A-4D. The other two factors, time of extraction and liquid-to-solid ratio were kept at 30 minutes and 30 to 1, respectively. The yield decreased as the methanol concentration increased from 70 to 90%, while the temperature showed a better response from 65°C to 75°C. This was in agreement with the results in Table 3, whereby the linear relationship in the reactions were significant [16].

The interactive influences of the extraction of methanol concentration to the liquid-to-solid ratio are provided in Figure 4C. The other two variables extraction temperature and time were kept at 70°C and 30 minutes, respectively. The liquid-to-solid ratio gave the best yield of flavonoids at 20 to 1 and decreased with an increase in the ratio. Figure 4D shows the effect of temperature on the time of extraction, with the methanol at 80% and liquid-to-solid ratio of 30. A high yield was obtained at 65°C and 75°C, with a slight dip at 70°C.

Thus, a temperature between 65 and 75°C may be used for the extraction at these conditions.

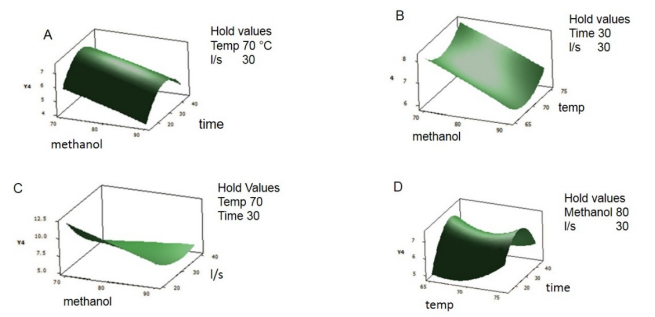

Figure 4: Surface plot of independent variable (A)Methanol against time, (B)Methanol against temp, (C)Methanol against l/s, (D)Temperature against time.

Source	S.S	df	M.S.	F-value	p-value prob>F
Regression	105.585	14	7.5418	10.09	0
Linear	65.438	4	16.3595	21.88	0
Methnol	8.036	1	8.036	10.75	0.007
Temp	0.15	1	0.1496	0.2	0.663
Time	0.832	1	0.8321	1.11	0.312
L/S	56.42	1	56.42	75.45	0
Square	30.756	4	7.6889	10.28	0.001
Methnol*methnol	0.043	1	0.0133	0.02	0.896
Temp*temp	3.221	1	1.904	2.55	0.137
Time*time	21.301	1	12.4033	16.59	0.002
L/s*l/s	6.192	1	6.192	8.28	0.014
Interaction	9.391	6	1.5652	2.09	0.13
Methnol*temp	0.018	1	0.0182	0.02	0.879
Methnol*time	0.036	1	0.0361	0.05	0.83
Methnol*l/s	7.102	1	7.1022	9.5	0.01
Temp*time	0.766	1	0.7656	1.02	0.332
Temp*l/s	0.292	1	0.2916	0.39	0.544
Time*l./s	1.177	1	1.1772	1.57	0.233
Residual Error	8.973	12	0.7478		
Lack-of-Fit	8.247	10	0.8247	2.27	0.344
Pure Error	0.727	2	0.3633		
Total	114.558	26			

Table 3: Analysis of Variance (ANOVA) for the experimental result.

The effect of methanol and time of extraction are shown in Figure 4A. Methanol concentration of 70% and a time of 30 minutes gave the best yield at 70°C and a liquid-to-solid ratio of 30 to 1.

Comparison of optimized maceration extraction method and Soxhlet extraction

Soxhlet extraction was performed with the temperature setting of 70°C. Four grams of sample powder was placed into a thimble. 80 mL of 70% methanol was used as solvent and the Soxhlet was let to run for 24 hours. The Soxhlet extraction has produced 65 mg/ml of total flavonoids, which is higher than that of the optimized maceration extraction (13.31 mg/ml). However, the optimized extraction method has an advantage of reduction in extraction time as only 30 mins is required for the mentioned yield and 5 more runs in the same period of time would give more yield than the Soxhlet extractor.

Conclusion

An optimized microwave-assisted extraction method of total plant crude extracts from *Cyanotis beddomei* has been developed with microwave assisted extraction. This is the first report on the extraction of total plant crude extract from *Cyanotis beddomei* using MAE. MAE and preliminary tests enabled us to screen several significant extraction factors and find out the optimum extraction conditions in a quick and economical way. Total plant crude extract can be efficiently extracted from *Cyanotis beddomei* using MAE. The statistical testing and the 3-D response surface plots indicated that the changes of liquid to solid ratio, extraction time and extraction temperature and methanol proportion of MAE had a significant effect on total plant extraction yield. The extraction yield of total plant extract was 13.31 mg/g under the optimization conditions at ethanol proportion of 70%, extraction time of 30 mins, extraction temperature of 70°C and ratio of liquid to solid of 20 mL/g.

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