

## EXTRACTION AND EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF *SPATHIPHYLLUM CANNIFOLIUM*

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### Graphical abstract

Run	Factor 1 A: temperature (°C)	Factor 2 B: Time (min)	Experimental result: IC <sub>50</sub> (µg/ml)
1	40	30	55.7
2	70	30	23.21
3	40	90	75.42
4	70	90	25
5	40	60	6.314
6	60	60	52.8
7	55	30	17.96
8	55	90	8.807
9	55	60	19.98
10	55	60	21.43
11	55	60	12.82

### Abstract

*Spathiphyllum cannifolium* is abundantly planted in Malaysia as a landscape plant. However, this plant also has medical benefit to human as it was previously found to possess anti-bacteria and anti-cancer activities. In the present study, the plant was evaluated for its anti inflammatory activity and the optimum condition for the extraction of bioactive compounds with anti inflammatory activity was determined. Semi purification of the compounds possessing anti inflammatory activity was conducted as well. To extract the bioactive compound, sonication with methanol as a solvent extraction was carried out. Face-Centered Central Composite Design (FCCCD) and Response Surface Methodology (RSM) were used to design and identify the optimum condition to extract the compound, respectively. The results showed that the plant was positive for the anti inflammatory activity with IC<sub>50</sub> 6.3 µg/ml. Sonication for 60 min at 40°C using medium frequency was the optimum condition to carry out the extraction for bioactive compounds. After semi purification of the initial crude extract by solvent partitioning, chloroform fraction was found to be positive for anti inflammatory activity. In conclusion, this study suggested that *S. cannifolium* possess anti inflammatory activity and should be further analyzed to identify the specific compounds that responsible for this activity.

**Keywords:** *Spathiphyllum cannifolium*, anti inflammatory, sonication, Face-Centered Central Composite Design (FCCCD)

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## 1.0 INTRODUCTION

*Spathiphyllum cannifolium* is a type of monocotyledonous flowering plants. It is originated from Southeastern Asia and America. In Malaysia, it was abundantly planted as a landscape plants. Previous studies showed this plant possess compounds that have medical benefit to human, such as, anti-bacteria and anti-cancer agents [1, 2]. Ahmad-Raus *et al.* (2013) [3] suspected flavonoids contain in this plant leaves is responsible for antibacterial activity. Isolated lipid from the areal part of *S. cannifolium* contain stigmasterol [4] which is classified under phytosterol and known to be effective in reducing the

risk of cancer such as colon, breast and prostate cancer [5]. In this study, we evaluated the potential of the plant to possess anti inflammatory activity.

Inflammation is a natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganism, toxic chemical substances and physical damage to tissue [6]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [7]. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils

and macrophages, from the blood into the injured tissues [8].

Another type was the chronic inflammation. Chronic inflammation happens when the acute inflammation last for over a long time. Sometimes, it also caused by certain hazardous pathogen that directly cause the chronic inflammation. Over a long time, chronic inflammation if not treated could result in loss function of certain part of body. The excessive production of nitric oxide (NO) in human body usually triggers the inflammation. This NO was generated, when it was induced by inducible nitric oxide (iNOS) [9]. Or, in other way, excessive production of prostaglandin (PGE) also leads to inflammation.

In order to encounter inflammation, there were many anti inflammation medicines have been invented. However, some issue arises with those non-steroidal anti-inflammation (NSAID) medications that cause harmful side effects such as gastrointestinal irritations [10]. Other types of anti-inflammatory medicines such acetaminophen can cause liver damage and, Cox-2 inhibitor Vioxx® and Celebrex® can cause heart problem [11]. So, there is a need to create other options to encounter this problem. This study is an effort to expand a variety of anti-inflammatory drugs other than the ones that are available in the market.

## 2.0 EXPERIMENTAL

### 2.1 Plant Collections and Pre-treatment

*S. cannifolium* leaves were collected from International Islamic University of Malaysia (IIUM) resident compound and the herb garden, Botanical Park, Kuala Lumpur. The plant was verified by a botanist and recorded in Department of Biotechnology Engineering, IIUM. The leaves were cut into small pieces and dried in the oven at 40°C for 3 days. After 3 days, the dried leaves were grinded until it becomes powder

### 2.2 Plant Extraction

The grinded *S. cannifolium* leaves were soaked with methanol to extract bioactive compounds from the plant. The ratio of the mass of sample to the volume of methanol is 1:10. In this study, extraction was conducted using ultrasonication at medium frequency (24 kHz). After sonication, the extract was filtered using Whatman paper and spun at 4000 rpm for 10 minutes. The resulting supernatant was collected without disturbing the sediments or pellet and the crude extract was obtained by the drying the supernatant in water bath at 50°C.

### 2.3 Optimization of Extraction

To identify the optimum sonication processing condition to extract the bioactive compounds with anti inflammatory activity, a series of experiment was

designed by Face-Centered Central Composite Design (FCCCD) which was available in Design Expert software 8.0.7.1 [12]. Table 1 shows the series of experiment with 11 runs and two parameters which were temperature and time, that were the manipulated variables of the extraction processing condition. The response of this study is the extract concentration at 50% of inflammatory inhibition (IC<sub>50</sub>). The ranges of temperature and time for this study were decided based on the previous plant extraction studies carried out in our lab [13].

### 2.4 Statistical Analysis

The analysis of the results of the optimization experiments was conducted using regression analysis. In this analysis, multiple regression equation using secondary-order polynomial model was developed and followed by analysis of the regression equation by statistical tools which included ANOVA (Analysis of Variance), F and P test. The second-order polynomial model for two factors before fitting the experimental data is shown by the equation below [14].

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{12}AB$$

Where Y is the dependent variable or response (extract concentration at 50% of inflammatory inhibition, IC<sub>50</sub>), A and B are independent variable (temperature and time, respectively);  $\beta_0$ : intercept;  $\beta_1$ ,  $\beta_2$ : linear coefficients;  $\beta_{11}$ ,  $\beta_{22}$ : squared coefficients;  $\beta_{12}$ : interaction coefficient.

The determination of coefficient, R<sup>2</sup>, which measures the quality of fit of the polynomial model, was also determined. The 3D response surface and 2D contour plots were examined to evaluate the model and to determine the optimum processing conditions.

### 2.5 Solvent Partitioning

The solvent partitioning starts with the least polar solvent, which was the n-hexane followed by chloroform, ethyl acetate and water. In separating funnel, n-hexane was added into the crude extract that had been dissolved with methanol. Adequate amount of water was then added to produce more significant immiscible layer. These mixtures were shaken up and down several times. After the immiscible layer can be seen clearly, the upper and the bottom part were separated into two separate containers. The containers were labeled with n-hexane fraction (top layer) and extract fraction (bottom layer), respectively. This step was repeated several times to increase the efficiency of separation process. After that, the bottom layer was transferred into a new separating funnel and sufficient amount of chloroform was added. The same procedure with n-hexane is repeated and this was followed by partitioning in ethyl acetate and eventually water. Thin layer chromatography (TLC) was conducted for each

solvent to determine the bioactive compounds contain in each solvent fraction.

## 2.6 Nitric Oxide (NO) Assay

Nitric oxide assay was first conducted by culturing macrophage cells, RAW 264.7 in 96-well plate for 12 hours followed by the addition of 50 µl of extract at varying concentration in each well, individually. The plate was incubated for 2 hrs before treatment with 50 µl lipopolysaccharide (LPS) at 4 µg/ml to stimulate the production of nitric oxide (NO) was conducted. After 24 hrs of LPS treatment, 50 µl of the cell supernatant

was transferred into a new well plate. Then, 50 µl (or equal volume of the supernatant) of Griess reagent was added to examine the NO concentration. The absorbance was measured using Thermo Scientific microplate reader at 540nm absorbance [15]. The absorbance was used to calculate the percentage of inflammatory inhibition using the equation below:

$$\% \text{ inhibition} = \frac{\text{absorbance (cell + lps)} - \text{absorbance (cell + lps + extract)}}{\text{absorbance (cell + lps)} - \text{absorbance (media)}}$$

## 3.0 RESULTS AND DISCUSSION

### 3.1 Analysis of Optimization Experiments

In the present study, FCCCD under the response surface methodology (RSM) had been employed for determining the optimum extraction condition since it is the most useful method for developing a second-order polynomial (quadratic) model which considers the effects of linear, quadratic, and interactions of the factors to the response [16]. The design of experiment and regression analysis for this optimization study was performed using the Design Expert (Version 6.0.8) statistical software. The most popular option for CCD is Face Centered Central Composite Design (FCCCD) where the axial point (alpha) is set to be 1.0 coded unit from the center point. By this method, three levels were obtained for the two factors involved in the optimization of the extraction process. The generated experimental runs were executed and the results are displayed in Table 1. Each run was performed in triplicate reading and the average of IC<sub>50</sub> obtained was taken as the experimental values of the dependent variable or response (Y).

From the experimental result in Table 1, it is shown that the best response was achieved when sonication extraction was conducted at 40°C for 60 min which resulted in 6.31 µg/ml of IC<sub>50</sub>. While sonication at 40°C for 90 min resulted in poor IC<sub>50</sub> of 75.42µg/ml. Regression analysis can be used to predict the best response within the experiment constraints using a second-order polynomial model to include the quadratic and interactions effects [17]. The second-order polynomial equation developed by the software in terms of coded factors is as the following:

$$\text{Log}_{10}(\text{IC}_{50}) = 1.14 + 0.010 \cdot A - 0.024 \cdot B + 0.025 \cdot A \cdot B - 0.27 \cdot A^2 + 0.11 \cdot B^2$$

Where A, and B are the independent variable (temperature, and time of extraction)

**Table 1** Experimental results for extract concentration at 50% of inflammatory inhibition (IC<sub>50</sub>) with two extraction factors; temperature and time; using Face Centered Central Composite Design (FCCCD)

Run	Factor 1 A: temperature (°C)	Factor 2 B: Time (min)	Experimental result: IC <sub>50</sub> (µg/ml)
1	40	30	55.7
2	70	30	23.21
3	40	90	75.42
4	70	90	25
5	40	60	6.314
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Before the equation above can be used to predict the best response, the adequacy of the model above was evaluated based on coefficient of R<sup>2</sup> and model p-value. In order to make it acceptable, the R<sup>2</sup> must be greater than 0.8 and the p-value must be less than 0.05. The ANOVA result of this study is presented in Table 2.

From Table 2, "Model F-value" of 0.33 implies the model is not significant relative to the noise. There is a 87.51 % chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this study, there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 18.38 implies there is a 5.20% chance that a "Lack of Fit F-value" this large could occur due to noise. Thus, this study showed lack of fit is poor. R<sup>2</sup> of this model is 0.248 indicating the model is not acceptable as the it should be greater that 0.8. Due to the analysis above, the second-order polynomial equation above cannot be used to plot the 3D response surface and 2D contour plots that is used to determine the optimum values of the variables within the ranges considered. The main target of response surface is to hunt efficiently for the optimum values of

the variables such that the response is the best. However, as the model is not significant, both plots cannot be plotted to predict the optimum values of the variables. Thus, to obtain the optimum processing condition to extract bioactive compounds with anti-inflammatory activity, it is decided to use the run that gives the best or the lowest IC<sub>50</sub>. From Table 1, the run that gives the lowest IC<sub>50</sub> is the extraction that is carried out at 40°C for 60 mins

**Table 2** ANOVA of the second-order polynomial equation

Source	Sum of Squares	Mean Square	F-Value	p-Value prob > F	
Model	0.2765	0.0553	0.3305	0.8751	NS
A (temp)	0.0006	0.0006	0.0039	0.9526	
B (time)	0.0035	0.0035	0.0211	0.8902	
AB	0.0024	0.0024	0.0147	0.9081	
A <sup>2</sup>	0.1829	0.1829	1.0935	0.3436	
B <sup>2</sup>	0.0289	0.0289	0.1728	0.6948	
Residual	0.8366	0.1673			
Lack of Fit	0.8074	0.2691	18.381	0.0520	NS
R- Squared					0.2484

NS=not significant

### 3.1 Analysis of Solvent Fractions

Solvent partitioning was conducted after the sonication extraction at 40°C for 60 mins was carried out. Using this condition, 500 g of crude extract was partitioned with hexane, chloroform, ethyl acetate and water, sequentially. TLC analysis was applied to each fraction to determine the bioactive compounds present in each fraction. Both, n-hexane and ethyl acetate fractions showed similar spot patterns with chloroform fraction. However, the observed spots for both n-hexane and ethyl acetate fractions were faint compared to chloroform fraction indicating less amount of the compounds present in both fractions. Thus, retention factor (R<sub>f</sub>) value was calculated only for the spots observed in the chloroform fraction (Table 3). No spot was observed on TLC plate of water fraction.

**Table 3** Retention factor and number of spots on TLC of chloroform fraction

Chloroform:Methanol (as mobile phase)	Number of Spots	R <sub>f</sub>
90:10	5	0.48
		0.56
		0.68
		0.87
		0.93

Nitric oxide assay was performed to evaluate the anti-inflammatory activity of chloroform and water fractions. From the assay, the IC<sub>50</sub> of chloroform fraction is 600.7 µg/ml and no IC<sub>50</sub> value in water fraction. Based on this result, only chloroform fraction will be used for further purification in the future to identify the exact compounds responsible for the anti-inflammatory activity.

## 4.0 CONCLUSION

In conclusion, *S. cannifolium* is a plant that possesses anti-inflammatory activity and further investigation is required before the plant can be developed as a potential source for anti-inflammatory drugs.

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