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# Computer-based estimation of antioxidant activity of *Caesalpinia sappan* L.

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## Introduction

*Caesalpinia sappan* L. is a plant that commonly known as Sappan wood, Brazil or East Indian red wood. It distributes in many parts of Asia such as India, Malaysia (Malay Peninsula), Thailand, etc. It has been used in food, beverages, and medicinal propose. The medicinal use of Sappan wood was recorded in Thai folk medicine,[1] Ayurveda,[2] Chinese tradition medicine[3] for treatment of diarrhea, dysentery, and skin infections.[4] Berger and Sicker suggested that Sappan wood contained red pigments used for histological staining.[5] The chemical composition of *C. sappan* heartwood are coumarin, chalcone, xanthone, flavone, isoflavone and brazilin. Brazilin (Figure 1) is a biologically active compound to antibacterial activity, anti-inflammatory, and antioxidant activity.[6]

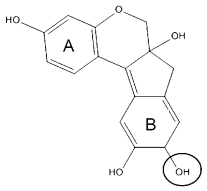
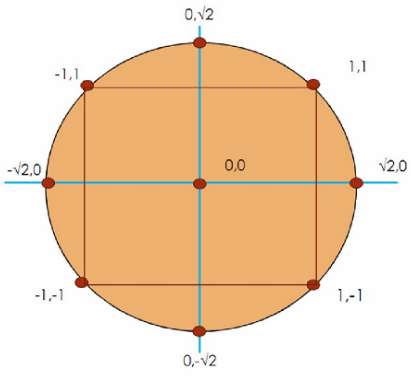



**Figure 1.** Brazilin chemical structure.

Normally, optimization experiment one factor is a time and this method is called one factor at a time. This technique is failed to accurate the interaction effect.[7] The optimal condition was optimized using design-expert program from commercial computer software to estimate the effect of multiple factor interactions on one or more response variables. In the previous study, *C. sappan* has been reported to be very beneficial in antioxidant activity.[8] The objective of this study was to estimate the antioxidant activity of *C. sappan* heartwood using computer software. The optimal condition providing the highest antioxidant activity was investigated.

## Methods

***Experimental design and plant extraction*:** The condition of extraction; temperature ($X_1$) and time ($X_2$) was designed based on spherical composite design. $X_1$ and $X_2$ were varied from 45-95 °C and 30-60 min, respectively. The model conditions are shown Figure 2. The two responses; half maximal inhibitory concentration ($IC_{50}$) obtained from DPPH assay ($Y_1$) and FRAP assay ($Y_2$) were monitored. Design Expert® software version 11.0 (Stat-Ease, Inc., USA) was used for the estimation study.




| Condition | $X_1$ | Temp. (°C) | $X_2$ | Time (min) |
|---|---|---|---|---|
| 1 | -1 | 52.3 | -1 | 34.4 |
| 2 | 1 | 87.7 | -1 | 34.4 |
| 3 | -1 | 52.3 | 1 | 55.6 |
| 4 | 1 | 87.7 | 1 | 55.6 |
| 5 | -√2 | 45 | 0 | 45 |
| 6 | √2 | 95 | 0 | 45 |
| 7 | 0 | 70 | -√2 | 30 |
| 8 | 0 | 70 | -√2 | 60 |
| 9 | 0 | 70 | 0 | 45 |
| 10 | 0 | 70 | 0 | 45 |

**Figure 2** Two-factor spherical composite experimental design

Dried *C. sappan* heartwood powder (50 g) and water (300 mL) was placed into a 500-mL Erlenmeyer flask. The flask was placed in a water bath and extracted using specific condition as showed in Figure 2.The mixture was filtered using Whatman No. 1 filter paper, the marc was extracted again for two times, and the pooled filtrate was lyophilized using freeze dryer (LGJ-18S, Scienergy solution Co., Ltd. China). The extracts were stored at -20 °C until use.

***DPPH radical scavenging assay***: The DPPH radical scavenging assay was determined by the method described by Brand-Williams et al.[9] The extract (100 µL) was mixed with equal volume of 200 µM of 2, 2-diphenyl-1-picrylhydrazyl. After 30 min incubation period in the dark at room temperature, the absorbance was measured compared to blank at the wavelength of 517 nm. The scavenging activity (%S) was calculated using the following equation: $\%S = ((A_{control} - A_{sample})/ A_{control}))\times100$, where $A_{control}$ is absorbance of blank control (containing all reagents except the extract solution) and $A_{sample}$ is absorbance of the test sample. The Results were expressed as $IC_{50}$.

***Ferric reducing power (FRAP) assay***:[10] The FRAP reagent was prepared by mixing acetate buffer (300 µM, pH 3.6), a solution of 10 µM TPTZ in 40 µM HCl and 20 µM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (270 µL) was mixed with extract solution (30 µL) before subjected to measure absorbance at 595 nm after the mixture was incubated for 30 min in the dark at room temperature. The reducing power (% inhibition) was calculated using the following equation: $\%Inhibition = ((A_{sample} - A_{control})/ A_{sample}))\times100$, where $A_{sample}$ is absorbance of the test sample and $A_{control}$ is absorbance of blank control (containing all reagents except the extract solution). Results were expressed as $IC_{50}$.

## Results

The experiment $IC_{50}$ values are shown in Table 1. This result could be used to estimate the antioxidation activity outside the design points.

**Table 1.** $IC_{50}$ values of the *C. sappan* in different extraction conditions

| Condition | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | DPPH assay | FRAP assay |
| 1 | 15.08±.055 | 6.31±0.08 |
| 2 | 13.11±0.73 | 6.31±0.32 |
| 3 | 46.59±0.11 | 7.93±0.04 |
| 4 | 53.61±0.19 | 7.07±0.07 |
| 5 | 23.09±1.09 | 6.73±0.30 |
| 6 | 20.79±1.81 | 7.23±0.27 |
| 7 | 26.88±3.51 | 6.87±0.17 |
| 8 | 20.07±0.53 | 5.94±0.10 |
| 9 | 21.90±4.42 | 6.57±0.57 |
| 10 | 22.37±0.94 | 5.58±0.16 |

The contour plots and response surfaces of the model condition are shown in Figure 3 and Figure 4, respectively. Results from DPPH assay showed that extraction temperature did not affect the $IC_{50}$ values. While decreasing of extraction time, the low $IC_{50}$ value achieved, indicating that antioxidant activity was high when used low extraction time. From visual observation, the maximum antioxidant activity achieved when used low extraction time for all extraction temperature. According to FRAP assay, the $IC_{50}$ value was high at low and high extraction temperature, the lowest $IC_{50}$ obtained at medium temperature. At low and medium extraction temperature, decreasing of extraction time, $IC_{50}$ also decreased. While, at high extraction temperature, decreasing of extraction time, $IC_{50}$ increased. From visual observation, the maximum antioxidant activity achieved when used medium extraction temperature as well as extraction time.

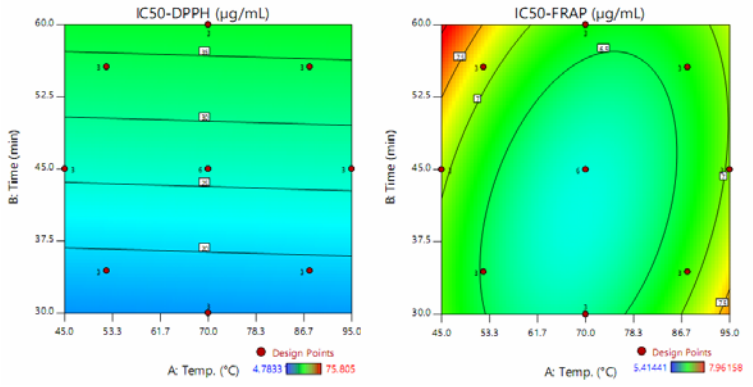



**Figure 3.** Contour plots of the $IC_{50}$ obtained from DPPH assay (left) and FRAP assay (right)

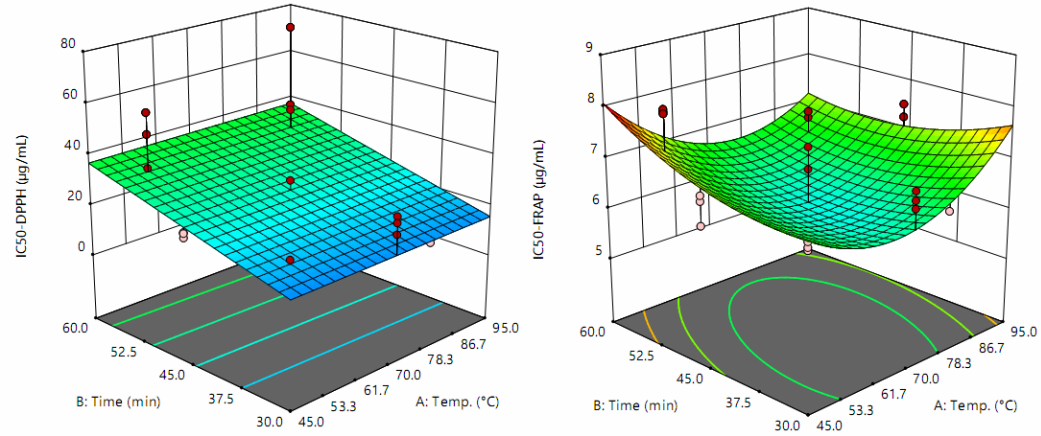



**Figure 4.** Response surfaces of $IC_{50}$ obtained from DPPH assay (left) and FRAP assay (right)

The highest antioxidant activity achieved with the lowest $IC_{50}$ value. The estimation from the computer software reported the model and estimation equation as showed in Table 2, which response of $IC_{50}$ value obtained from DPPH assay and FRAP assay was linear and quadratic model, respectively. Furthermore, it also reported that the optimal condition provided the lowest $IC_{50}$ value from simultaneous two assays was extraction temperature of 65.6 °C and extraction time of 32.82 min with the desirability of 0.753. The optimal condition provided the $IC_{50}$ value of 17.34 and 6.21 µg/mL, respectively.

**Table 2.** Model and equation for estimation of $IC_{50}$ of *C. sappan* obtained from different methods

| Response | Model | Equation |
|---|---|---|
| $IC_{50}$-DPPH ($Y_1$) | Linear | $Y_1 = -7.621+0.013(X_1)+0.735(X_2)$ |
| $IC_{50}$-FRAP ($Y_2$) | Quadratic | $Y_2 = 12.731-0.160(X_1)-0.054(X_2)-0.001(X_1)(X_2)+0.002(X_1)^2+0.002(X_2)^2$ |

$X_1$ = temp., $X_2$= time

## Discussion

Extraction condition was found to be an essential factor to enhance efficiency of extraction. Several research reported that thermal treatments are the main cause of the depletion of natural antioxidant activity.[11-12] The oxidative heat damage was also reported by Zanono et al.[13] who found ascorbic acid was decreased when temperature increased. Azman Abdul Rahim et al.[14] reported that time is a very important factor for extraction. Our results showed that, the extraction condition offer the highest antioxidant activity used medium extraction temperature as well as extraction time. Furthermore, Majeed et al. reported that study phenolic compound a powerful scavengers of free radicals as displayed a good correlation between total phenolic compound and DPPH radical scavenging activity. It was discussed that the phenolic content advocate to overall antioxidant activities of extracts.[15]

## Conclusion

Computer software was used to estimate the antioxidant activity of *C. sappan*. This work success in DPPH assay and FRAP assay. When extraction temperature of 65.6 °C and extraction time of 32.82 min provided that highest antioxidant activity of *C. sappan* extract. The $IC_{50}$ values of 17.34 and 6.21 µg/mL were achieved from the optimal condition. finding the extraction condition provided the highest antioxidant activity from simultaneously two assays;

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