Analysis of enzymatic extraction of saponin
by experiment design methodology

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Abstract. Sapindus mukorossi (soapberry) is widely distributed in Taiwan, India, China and Japan. Its fruit contain saponin, which consist of two main part, the aglycone (sapogenin) and the polysaccharides (sugar moiety) which connected through glyosidic linkage. It has many pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, etc. Thus, this plant become one of high added value bioresources for pharmaceutical field. There are many methods to extract saponin. In this experiment, enzyme extraction is used to extract the saponin that known as high efficiency and specify method. Then, we analyzed its by-product, total polysaccharides content and its antioxidant properties through total polyphenol content. With Response Surface Methodology (RSM), we obtained the optimum condition for the best total saponin, total polysaccharides content, and total polyphenol content. Based on RSM analyses, with solid-liquid ratio of 1:5, extraction time of 3 hours, and enzyme concentration of 0.55%, we obtained the total saponin is 115.794 mg/mL, the total polysaccharides is 155.3 mg/mL and total polyphenol content is 1.309 mg/mL.

1. Introduction
Sapindus mukorossi is a genus of Sapindus and known as soapberry, and widely distributed in Asia region such as Taiwan, India, China and Japan. [1] The main chemical components of this plant are saponin, fatty oil and protein. Saponin is mainly found in the pericarp of the saponin, and it is a non-ionic surfactant especially for good foaming and stain removal properties. It consists of two main part, the aglycone (sapogenin) and the polysaccharides (sugar moiety) which connected through glyosidic linkage. [2] It has many pharmacological and biological properties such as exhibit molluscicidal effect against the golden apple snails [3], anti-fungal [4], anti-cancer, antimicrobial, anti-tyrosinase, antioxidant [5], anti-inflammatory, hepatoprotective activity, anxiolytic activity, anti-bacterial [6] and other physiological effects. Thus, this plant become one of promising resources for pharmaceutical field especially for cosmetic and medicine application.

Many methods have been done to extract this saponin such as water extraction based fermentation [7], ultrafiltration [8], foam fractionation [9], organic solvent extraction [3], etc. Those methods are found complex and difficult to be applied at industrial scale even harmful for environment because of using...
organic solvent. Therefore, using enzyme extraction can be the alternative way in order to obtain high quality of saponin that might be applied into industrial scale. The enzyme is highly efficient and accurate, and can cause different degrees of changes in the plant cell wall, such as softening, swelling and collapse, thereby changing the permeability of the cell wall, and improving the extraction rate of the drug effect. Moreover, the enzyme can be possible apply for industrial scale.

In this experiment, the pericarp of soapberry is extracted using the enzyme extraction. Some factors, such as solid-liquid ratio, enzyme concentration, and reaction time will be analyzed and optimized using Response Surface Methodology (RSM) as a tool to find the optimal response within specified range of the factors. With RSM, it is simultaneously optimize the levels of these variables to attain the best performance system for obtaining total saponin, total polysaccharides content, and total polyphenol content. Thus, by finding the optimum parameters of enzyme extraction can increase the economic value of the soapberry.

2. Experiment

The enzymatic extraction process was conducted in the water extraction with using enzyme cellulase, and the solid-liquid separation was carried out by adding to the centrifuge for 15 minutes, and the supernatant was taken and stored at 4°C for further analysis. Before design the experimental using RSM, one-factor-at-a-time test was conducted to find the optimal factor level range of temperature, time, solid-liquid ratio and enzyme concentration of each factor. Through a one-factor-at-a-time test, only one factor is moved at a time, while other factors maintain at the level of the previous experiment to analyses the effect of the factor level shifting. Thus, the selection of the optimum factor level range from each factors are carried out. Based on Figure 1, as the temperature increase, the total saponin also increase while for the extraction time over 3 hours, the total saponin decrease. Figure 2 shows difference solid liquid ratio in which using 1:5 gave highest saponin content.

![Figure 1. Effect of temperature and time.](image1)

![Figure 2. Effect of Solid-liquid ratio and time.](image2)

The response surface methodology (RSM) was conducted in this study for the experimental design in 3 factors and 3 levels. The 3 factors for the enzymatic hydrolysis process were selected as time (X₁), solid-liquid ratio (X₂), enzyme concentration (X₃) and the response were the total saponin extract (Y₁), total polysaccharides content (Y₂) and total polyphenol content (Y₃). The 3 levels (-1, 0, +1) of the 3 factors were listed in Table 1 and the analysis of the variance method was applied to discuss the effects of each variable and the interaction between variables.

| Level | X₁ (hour) | X₂ (w/v) | X₃ (%) |
|-------|-----------|----------|--------|
| -1    | 1         | 5        | 0.1    |
| 0     | 2         | 10       | 0.55   |
| +1    | 3         | 15       | 1.0    |
3. Results and Discussion

3.1. Total saponins content (mg/mL)

There were 15 experimental runs designed by Box-Behnken experimental design methodology for the enzymatic hydrolysis process of this study and the total saponin extract ($Y_1$) were obtained and listed in Table 2. The 14th run with maximum total saponin extract of 115.8 mg/mL was obtained in the operating factors of time ($X_1$) at 3 hours, solid-liquid ratio ($X_2$) at 1:5, and enzyme concentration ($X_3$) at 0.55%.

Table 2. The total saponin, polysaccharide content, polyphenol extracts in the 15 experimental Runs of RSM.

| Run | Mode | $X_1$ | $X_2$ | $X_3$ | $Y_1$ (mg/mL) | $Y_2$ (mg/mL) | $Y_3$ (mg/mL) |
|-----|------|------|------|------|--------------|--------------|--------------|
| 1   | −0+  | 1    | 10   | 1    | 75.31        | 66.65        | 0.740        |
| 2   | 000  | 2    | 10   | 0.55 | 86.75        | 89.59        | 0.807        |
| 3   | 0++  | 2    | 15   | 0.1  | 70.21        | 23.40        | 0.566        |
| 4   | −0−  | 1    | 5    | 0.55 | 110.4        | 138.5        | 1.251        |
| 5   | 0−+  | 2    | 5    | 1    | 109.8        | 153.1        | 1.281        |
| 6   | 000  | 2    | 10   | 0.55 | 86.46        | 81.22        | 0.732        |
| 7   | +0−  | 3    | 10   | 0.1  | 80.25        | 74.48        | 0.741        |
| 8   | −0−  | 1    | 10   | 0.1  | 80.61        | 50.99        | 0.720        |
| 9   | −+0  | 1    | 15   | 0.55 | 65.27        | 47.27        | 0.566        |
| 10  | +0+  | 3    | 15   | 0.55 | 67.90        | 11.16        | 0.520        |
| 11  | 0++  | 2    | 15   | 1    | 64.85        | 14.72        | 0.522        |
| 12  | 0−−  | 2    | 5    | 0.1  | 105.6        | 135.6        | 1.257        |
| 13  | +0+  | 3    | 10   | 1    | 83.82        | 65.41        | 0.731        |
| 14  | −0−  | 3    | 5    | 0.55 | 115.8        | 155.3        | 1.309        |
| 15  | 000  | 2    | 10   | 0.55 | 94.84        | 54.40        | 0.797        |

The total saponin extract ($Y_1$) in the 15 runs and their operating factors were regressed to obtain a second-order polynomial equation as follow.

$$Y_1 = 60.35 + 2.036 \times (X_1) + (-21.65) \times (X_1 - 2) + (-21.65) \times \left(\frac{X_1 - 2}{5}\right) + (0.316) \times \left(\frac{X_2 - 0.4}{0.4}\right) + (0.316) \times \left(\frac{X_3 - 0.4}{0.4}\right) + (X_1 - 2) \times \left(\frac{X_2 - 0.4}{0.4}\right) + (X_1 - 2) \times \left(\frac{X_3 - 0.4}{0.4}\right) + (X_1 - 2) \times (-0.7) + (X_2 - 2) \times (X_3) + (X_2 - 2) \times (X_3 - 0.7) + (X_2 - 2) \times (X_3 - 0.7) + (X_2 - 2) \times \left(\frac{X_3 - 2}{5}\right)$$

The experimental results of the total saponin extract and the predictions from Equation 1 were plotted in Figure 3. It can be found that most of the predictions are coincident with the experimental data and the $R^2$ of the above regression equation is 0.98.

![Figure 3. Comparison of the predicted and experimental data.](image1)

![Figure 4. Effect of each variable on the response.](image2)

The effects of the factors on the response $Y_1$ can be identified in Table 3. The test was applied to identify the significance of the effect of each factor or the interaction between factors. In usual, the P-value will be reduced with the increase of t ratio. The smaller of the P-value is, the effect of the factor on the response will be more significant. From the results in Table 3, the P-values of solid-liquid ratio ($X_2$) is smaller than 0.05. It represents the...
The effect of each variable on the response of the total saponin extract was shown in Figure 4. The maximum total saponin extract were obtained of X1 at 2 hours and X3 at 0.55%, and X2 is gradually decrease.

3.2. Total polysaccharides content (mg/mL)

There were 15 experimental runs designed by Box-Behnken experimental design methodology for the enzymatic hydrolysis process of this study and the total polysaccharides content (Y2) were obtained and listed in Table 2. The 14th run with maximum total polysaccharide content of 155.3 mg/mL was obtained in the operating factors of time (X1) at 3 hours, solid-liquid ratio (X2) at 1:5, and enzyme concentration (X3) at 0.55%.

The total polysaccharides content (Y2) in the 15 runs and their operating factors were regressed to obtain a second-order polynomial equation as follow.

\[
Y_2 = 75.07 + 0.369 \times (X_1 - 2) + (-6.073) \times \left(\frac{X_2 - 1}{2}\right) + (1.921) \times \left(\frac{X_3 - 0.55}{0.45}\right) + (X_1 - 2) \times \left(\frac{X_2 - 1}{2}\right) + \left(\frac{X_3 - 12}{5}\right) \times (-13.24) + \left(\frac{X_1 - 2}{5}\right) \times (-6.18) + \left(\frac{X_2 - 1}{2}\right) \times \left(\frac{X_3 - 0.55}{0.45}\right) \times (-6.536) + \left(\frac{X_1 - 2}{5}\right) \times \left(\frac{X_3 - 12}{5}\right) \times \left(\frac{X_3 - 18}{5}\right) \times \left(\frac{X_3 - 18}{5}\right) \times (-8.33)
\]

The experimental results of the total polysaccharides content and the predictions from Equation 2 were plotted in Figure 5. It can be found that most of the predictions are coincident with the experimental data and the R2 of the above regression equation is 0.97.

The effects of the factors on the response Y2 can be identified in Table 4. The test was applied to identify the significance of the effect of each factor or the interaction between factors. In usual, the P-value will be reduced with the increase of t ratio. The smaller of the P-value is, the effect of the factor on the response will be more significant. From the results in Table 4, the P-values of solid-liquid ratio (X2) is smaller than 0.05. It represents the significant effect of X2 factors is significant and the order of the effect of these three factors on the response are X2>X1>X3. It also can be observed from Table 3, the interaction between factors are not significant.

### Table 3. Effect of variables on the total saponin extract.

| Term      | Estimate | Error  | t Ratio | P-value   |
|-----------|----------|--------|---------|-----------|
| Intercept | 89.35    | 2.091  | 47.73   | <.0001*   |
| X1        | 2.027    | 1.281  | 1.58    | 0.1743    |
| X2        | -21.65   | 1.281  | -16.91  | <.0001*   |
| X3        | -0.361   | 1.281  | -0.28   | 0.7890    |
| X1X2      | -0.699   | 1.811  | -0.39   | 0.7155    |
| X1X3      | 2.217    | 1.811  | 1.22    | 0.2753    |
| X2X3      | -2.388   | 1.811  | -1.32   | 0.2444    |
| X1X1      | -3.554   | 1.885  | -1.89   | 0.1180    |
| X1X2      | 4.041    | 1.885  | 2.14    | 0.0849    |
| X2X3      | -5.795   | 1.885  | -3.07   | 0.0276*   |

*Significant
The effect of each variable on the response Y3 can be identified in Table 5. The test was applied to identify the significance of the effect of each factor or the interaction between factors. In usual, the P-value will be reduced

\[
Y_3 = 0.779 + 0.003 \times (X1 - 2) + (-0.385) \times \left(\frac{X2 - 10}{5}\right) + (-0.001) \times \left(\frac{X3 - 1.55}{0.45}\right) + (X1 - 2) \times \left(\frac{X2 - 10}{5}\right) + (-0.017) + (X1 - 2) \times (X1 - 2) \times (-0.021) + \\
\left(\frac{X2 - 10}{5}\right) \times \left(\frac{X3 - 1.55}{0.45}\right) + \left(\frac{X1 - 2}{5}\right) \times (X2 - 10) \times (X3 - 1.55) \times (-0.025)
\]

The experimental results of the total polyphenol content and the predictions from Equation 3 were plotted in Figure 7. It can be found that most of the predictions are coincident with the experimental data and the $R^2$ of the above regression equation is 0.99.
with the increase of t ratio. The smaller of the P-value is, the effect of the factor on the response will be more significant. From the results in Table 5, the P-values of solid-liquid ratio (X2) is smaller than 0.05. It represents the effect of X2 factors is significant and the order of the effect of these three factors on the response are X2>X1>X3. It also can be observed from Table 5, the interaction between factors are not significant.

| Term       | Estimate | Standard Error | t Ratio | P-value  |
|------------|----------|----------------|---------|----------|
| Intercept  | 0.779    | 0.015          | 51.05   | <.0001*  |
| X1         | 0.003    | 0.009          | 0.32    | 0.7635   |
| X2         | -0.365   | 0.009          | 39.12   | <.0001*  |
| X3         | -0.001   | 0.009          | -0.14   | 0.893    |
| X1X2       | -0.026   | 0.013          | -1.97   | 0.1056   |
| X1X3       | -0.008   | 0.013          | -0.57   | 0.5907   |
| X2X3       | -0.019   | 0.013          | -1.27   | 0.2589   |
| X1X1       | -0.021   | 0.014          | -1.49   | 0.1961   |
| X2X2       | 0.153    | 0.014          | 11.13   | <.0001*  |
| X2X3       | -0.025   | 0.014          | -1.83   | 0.1273   |

*Significant

The effect of each variable on the response of the total polyphenol content was shown in Figure 8. The total polyphenol content was obtained at X2 is gradually decrease, the X1 and X3 are not significant.

4. Conclusion

In this study, the response surface methodology was conducted for the enzymatic extraction of Sapindus mukorossi to discuss the operating parameters and the optimum operating conditions. Based on the experimental data, the best total saponin extract was obtained in 115.8 mg/mL (X1 = 3 hr, X2 = 1:5, X3 = 0.55%). The effects of time (X1), solid-liquid ratio (X2) and enzyme concentration (X3) on the response of total saponin extract (Y1) was significant. Followed by obtaining total polysaccharides content in 155.3 mg/mL and total polyphenol content in 1.309 mg/mL by using the same optimum condition. From this study, Sapindus mukorossi contains high saponin than polyphenol content although both compound are known as secondary metabolites. But these two compounds can be potentially used as natural antioxidant resources.

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