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# Isolation of Total Saponins from *Sapindus mukorossi* Gaerth

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The study has been carried out to investigate the effects of single factors such as solvents extraction temperature, times, solid-liquid ration, and the time of extractions on the yields of saponins present in the pulp of *Sapindus mukorossi* Gaerth. On this basis, an L9 orthogonal design of experiment was adopted to determine the optimal conditions for the extraction of saponins. The factors that influence the extraction of saponins are put in the order of extraction times, extraction time, solid-liquid ration, and the best combination is that the powder of the pulp is extracted with EtOH (solid-to-solvent ratio = 1:8, w/v) for three times at 60˚C for 3 hours. Under these conditions, about 1.63 g saponins will be extracted from 10 g raw material. The stability test showed that the *Sapindus mukuross* saponins can maintain surface activity at water temperature (25˚C - 40˚C), pH (6.3 - 7.7) and water hardness (50 - 250 mg·L$^{-1}$). It is proved that *Sapindus mukuross* saponins are quality non-ionic active agent.

*Keywords*: Saponins; *Sapindus mukorossi* Gaerth; Orthogonal Design; Extraction

## Introduction

The species of the genus of Sapindus belonging to the Sapindaceae, has about five to twelve species of shrubs and small trees (FRPS, 1998). Members of the genus are commonly known as soapberries or soapnuts because the fruit pulp is used to make soap (FRPS, 1998). There is about 10% saponins in fruit pulp (FRPS, 1998), which makes it an ideal resource for the extraction of Saponins. Saponin, a natural non-ionic surfactant, not only has a good emulsifying, separating and dispersing capability but also is a good foam stabilizer with a great cleaning capacity (Zhang et al., 1993). Thus, it can be used as foam stabilizer for the building concrete (Lin, 1977), pesticide synergist (Hong & Tokunaga, 2000), and it also has antiviral, reducing blood pressure functions (Huang et al., 2007; Ibrahim et al., 2006, 2008; Kuo et al., 2005; Yukiyoshi, 2001; Yata et al., 1986), so it is widely used in daily chemical industries, building materials, food industries and agriculture.

Usually, saponin is extracted by using solvent like water, ethanol and methanol (Huang et al., 2008; Rao & Sang, 2006). Accordingly, this experiment will use ground pulp of *Sapindus mukorossi* as raw material. Meanwhile the impact brought about by different solvent, extraction times, extraction durations and different solid-liquid ratios has been investigated.

## Materials and Methods

### Plant and Chemical Material

Fruits pulps of *S. mukorossi* were collected from Tiantai of Zhejiang Province, China, in November 2008. Prior to all extractions, fruits pulp was dried at 60˚C for 48 h and was ground in a Wiley mill to pass a 0.5 mm poresize screen. Chromatograph solvents used during the study were of HPLC grade and the other solvents and reagents used during the study were of AR grade.

### Extraction Process

The main factors that affect the extraction of saponins like extraction solvents, temperature, time, times and materials ratio (weight of the fruit pulp: volume of the extracting solvent), were studied individually. The optimum extraction conditions were determined by $L_9(3^4)$ orthogonal design of experiments i.e. three levels and three different parameters.

### Estimation of Total Saponins

Saponins sample was collected from extraction solution with labware. Saponins concentration was measured in sample by HPLC (column: SymmetryTM C18 (3.9 mm i.d × 150 mm), 40˚C, Flowing phase: $CH_3CN$:$H_2O$ ($H_2O$:90% → 20%, 30 min), 1ml·min$^{-1}$; detection wavelength: 210nm).

### Stability Study

This study was carried out at vary water temperature (25, 35, 40˚C), pH (6.3, 7.0, 7.7), water hardness (50, 100, 250 mg·L$^{-1}$) with aqueous solutions having a saponin content of 4%. The Cmc and $\gamma$Cmc were determined by dynamic tension meter.

### Statistical Analysis

The results are expressed as means ± SD unless otherwise

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stated. The evaluation of statistical significance was determined by the one-way ANOVA test, these analyses were done with SPSS for WINDOWS, version 19.0, and with small letter < 0.05, capital letter < 0.01 considered to be statistically significant.

## Results and Discussion

Saponins in *S. mukorossi* Gaerth were extracted by nine solvents, and its content was measured by HPLC. The best extracting solvents was ethanol which can result in the high productivity of saponin, good quality of saponin colour and volatiles (**Table 1**).

**Figure 1** showed the yields of saponins tended to increase with a rise in the temperature range from 20˚C to 50˚C. It may be probable that the greater speed of the molecule movements in higher temperature so that saponins diffused more quickly from cell to extracting agent. But the yields of saponins could be slight changed temperature of surpassing 60˚C. Temperature's effect on extraction is dual. On one hand, higher temperature can accelerate the solvent flow and thus increase the saponins content and on the other hand, higher temperature can decrease the fluid density that may reduce the extraction efficiency. Hence, it was found that 60˚C was the optimum temperature for extracting the saponins.

**Figure 2** showed the yields of saponins extracted was minimum at 1:6 materials ratio. Further increase in the material ratio leads to slight changed in the yields of saponins. This phenomenon might be due to the fact that when the material ratio reached a certain level, the extract has well dissolved in the solution that may lead the contents of the extract become saturated and prevent further increase.

The yields of saponins extracted for 4 h reached maxima and prolonged extraction may not increasing yield (**Figure 3**). This increase in the saponins content may be due to the synergistic effect of other parameters involved.

The yields of saponins obviously increased with the no. of extractions from 1 time to 2 times. But the yields of saponins could be slight changed when the no. of extractions surpassing 2 times (**Figure 4**).

The parameters and the orthogonal design of experiment for the extraction of saponins were given in the **Tables 2** and **3**.

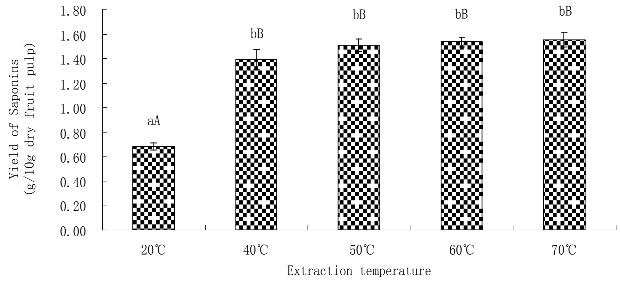


**Figure 1.**
Effects of temperature on the yield of *S. mukorossi* Gaerth. Saponins. Ethanol, solid-liquid ratio 1:20, time 6 h, 2 extraction times.

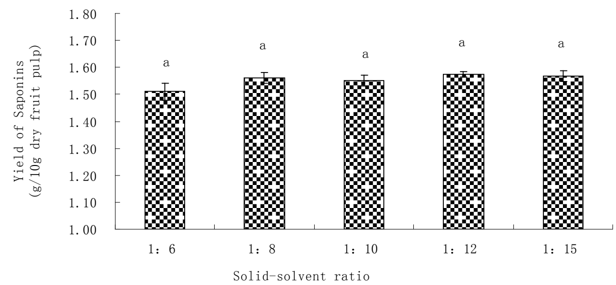


**Figure 2.**
Effects of the solid -solvent ratio on the yield of *S. mukorossi* Gaerth. Saponins. Ethanol, temperature 60˚C, time 3 h, 2 extraction times.

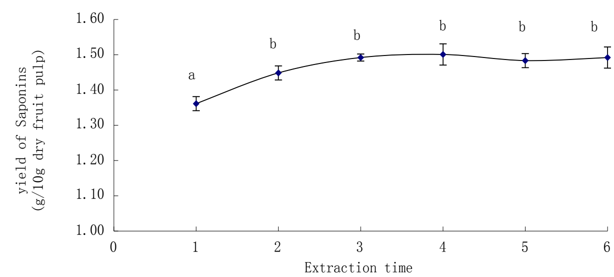


**Figure 3.**
Effects of extract time on the yield of *S. mukorossi* Gaerth. Saponins. Ethanol, temperature 60˚C, solid-liquid ratio 1:10, 2 extraction times.

**Table 1.**
Effects of solvents on the characteristics of *S. mukorossi* Gaerth. Saponins (means ± SD).

| | Extracting solvents | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MeOH | EtOH | Acetone | Butanol | $H_2O$ | 95% MeOH | 95% EtOH | 95% Acetone | 95% Butanol |
| Mass of Saponins (g) | 0.68 ± 0.12 | 1.54 ± 0.09 | 1.00 ± 0.12 | 0.98 ± 0.02 | 0.63 ± 0.11 | 1.19 ± 0.09 | 1.51 ± 0.06 | 1.37 ± 0.10 | 1.45 ± 0.07 |
| Purity (%) | 63.62 | 72.63 | 73.48 | 69.90 | 35.03 | 59.15 | 70.48 | 72.42 | 67.80 |
| Desiccation situation | Easy | Easy | Easy | Easy | Difficult | Little viscosity | Easy | Easy | Easy |
| Characters of the dry substance | yellow powder | Off-white powder | Light yellow powder | Light yellow powder | Brown glue | Yellow powder | Off-white powder | Off-white powder | Light yellow powder |
| volatiles | Slightly sweet | special fragrance | Special fragrance | special fragrance | Heavily sweet | slightly sweet | special fragrance | special fragrance | Special fragrance |

Note: Temperature 60˚C, solid-liquid ratio 1:20, time 6 h, 2 extraction times.

**Table 2.**
Factors and levels of orthogonal test.

| Levels | Extraction times (A) | Solid-liquid ration (B) | Extraction time (C) |
|---|---|---|---|
| 1 | 1 | 1:8 | 2 |
| 2 | 2 | 1:9 | 3 |
| 3 | 3 | 1:10 | 4 |

**Table 3.**
Design and result of orthogonal test.

| Test No. | A | B | C | Yield of *S. mukorossi* Gaerth. Saponins (g) |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1.05 |
| 2 | 1 | 2 | 2 | 1.34 |
| 3 | 1 | 3 | 3 | 0.92 |
| 4 | 2 | 1 | 2 | 1.48 |
| 5 | 2 | 2 | 3 | 0.89 |
| 6 | 2 | 3 | 1 | 1.29 |
| 7 | 3 | 1 | 3 | 1.54 |
| 8 | 3 | 2 | 1 | 1.57 |
| 9 | 3 | 3 | 2 | 1.61 |
| k1 | 1.04 | 1.36 | 1.30 | |
| k2 | 1.22 | 1.20 | 1.41 | |
| k3 | 1.57 | 1.27 | 1.12 | |
| R | 0.54 | 0.08 | 0.29 | |

**Table 4.**
Influence of different factors on the surface activity of *Sapindus mukuross* saponins.

| | Temperature (˚C) | | | Water hardness ($mg \cdot L^{-1}$) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25 | 35 | 40 | 50 | 100 | 250 | 6.3 | 7.0 | 7.7 |
| Cmc ($mg \cdot L^{-1}$) | 32.9 | 24.8 | 21.6 | 39.3 | 56.8 | 76.9 | 33.4 | 60.4 | 76.8 |
| $\gamma_{Cmc}$ ($mN \cdot m^{-1}$) | 36.3 | 35.2 | 34.2 | 35.9 | 36.1 | 37.4 | 36.5 | 37.6 | 38.2 |

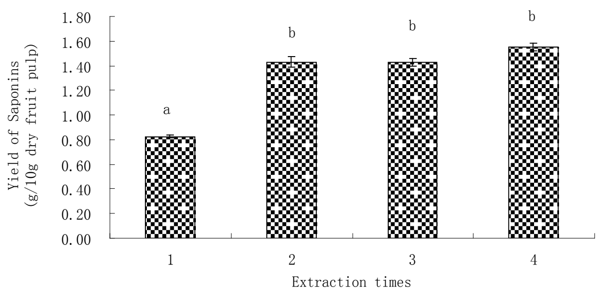


**Figure 4.**
Effects of extraction times on the yield of *S. mukorossi* Gaerth. Saponins. Ethanol, temperature 60˚C, solid-liquid ratio 1:10, time 3 h.

The factors that influence the extraction of saponins are put in the order of extraction times, extraction time, solid-liquid ratio, and the best combination is when the powder of the pulp is extracted with EtOH (solid-to-solvent ratio = 1:8, w/v) for three times at 60˚C for 3 hours. Under these conditions, about 1.63 g saponins will be extracted from 10 g raw material.

The results show that the *Sapindus mukuross* saponins can maintain surface activity at water temperature (25˚C - 40˚C), pH (6.3 - 7.7) and water hardness (50 - 250 $mg \cdot L^{-1}$) (**Table 4**).

## Conclusion

When the powder of the pulp was extracted with EtOH (solid-to-solvent ratio = 1:8, w/v) for three times at 60˚C for 3 hours, namely in the best extraction condition, the largest yield of saponins (1.63 g saponins will be extracted from 10 g raw material) was obtained. The stability test showed that the *Sapindus mukorossi* saponins can maintain surface activity at water conditions under which people normally use detergent. It is proved that *Sapindus mukorossi* saponins are quality non-ionic active agent. Thus, we can conclude that this technology for saponins extraction from *S. mukorossi* Gaerth. is efficient and environmentally friendly.

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## REFERENCES

Editorial Committee of the Flora of China of Chinese Academy of Science (1998) *Flora of China*. Beijing: Beijing Science Press.

Zhang, M. J., Liu, P. R., Zhao, J. Z., et al. (1993). Study on the comprehensive utilization of *Sapindus mukorssi*. *Natural Product Research and Development, 5*, 76-78.

Lin, Q. S. (1977). The chemical composition of Chinese herbal medicine. Beijing: People's Medical Publishing House.

Hong, K. J., & Tokunaga, S. (2000), Extraction of heavy metals from MSW incinerator fly ash using saponins. *Chemosphere, 41,* 345-352. http://dx.doi.org/10.1016/S0045-6535(99)00489-0

Ibrahim, M., Khan, A. A., Tiwari, S. K., et a1. (2006). Antimicrobial activity of Sapindus mukorossi and Rheum emodi extracts against *H. pylori*: *In vitro* and *in vivo* studies. *World Journal of Gastroenterology, 14,* 7136-7142.

Kuo, Y. H., Huang, H. C., Yang Kuo, L. M., et al. (2005). New dammarane-type saponins from the galls of *Sapindus mukorossi*. *Journal of Agricultural and Food Chemistry, 53,* 4722-4727. http://dx.doi.org/10.1021/jf047963s

Ibrahim, M., Khaja, M. N., Aara, A., et al. (2008). Hepatoprotective activity of *Sapindus mukorossi* and *Rheum emodi* extracts: *In vitro* and *in vivo* studies. *World Journal of Gastroenterology, 14,* 2566-2571. http://dx.doi.org/10.3748/wjg.14.2566

Yukiyoshi, T. (2001). The spermicidal affection of sapindus. *Natural Medicines, 55,* 563-568.

Yata, N., Sugihara, N., Yamajo, R., et al. (1986). Enchanced small intestinal absorption of β-lactam antibiotic in rats in the presence of monodesmosides isolated from pericarps of *Sapindus mukorossi* (Enmei-hi). *Journal of Pharmacobio-Dynamics, 9,* 211-217. http://dx.doi.org/10.1248/bpb1978.9.211

Huang, H. C., Tsai, W. J., Law, C. C., et al. (2007). Anti-platelet Aggregation *Triterpene saponins* from the galls of *Sapindus mukorossi*. *Chemical and Pharmaceutical Bulletin, 55,* 1412-1415. http://dx.doi.org/10.1248/cpb.55.1412

Huang, H. C., Wu, M. D., Tsai, W. J., et al. (2008). *Triterpenoid saponins* from the fruits and galls of *Sapindus mukorossi*. *Phytochemistry, 69,* 1609-1616. http://dx.doi.org/10.1016/j.phytochem.2007.10.033

Rao, H. Z., & Sang, C. T. (2006). Microwave-assisted extraction technology of sapindus-saponin. *Journal of Liaoning University of Petroleum & Chemical Technology, 26,* 70-72.