Effects of Different Irradiation Treatments on Total Saponins Content of Sapindus mukorossi

Yu-Min Shi¹, Heng Yan², Lin-Sen Wu¹, Jia-Jia Xie¹, and Hong-Guo Chen¹

Abstract
Sapindus mukorossi Gaertn is also known as Mu Huanzi, You Huanzi, soap tree, etc. The pericarp of Sapindus mukorossi contains many saponins, which is a type of natural non-ionic surfactant. Its extract has vigorous surface activity and biological activities such as bacteriostasis, oxidation resistance, and free radical scavenging. The Sapindus mukorossi extract is an environmentally friendly washing product that microorganisms can be rapidly decompose in nature without any environmental pollution. This study aims to investigate the effects of E-beam and Co⁶⁰-γ irradiation on the total saponins content in the crude extract of the S mukorossi. The S mukorossi powder is irradiated with E-beam and Co⁶⁰-γ ray at doses of 0, 4, 6, 8, 10, and 12 kGy for E-beam and 0, 50, 100, 150, and 200 Gy, respectively, for Co⁶⁰-γ ray. The changes in the content of total saponins in the crude extract, total detergency, and the bacteriostatic abilities before and after the irradiation were analyzed. The results showed that the content of total saponins in samples irradiated by E-beam was significantly higher than that in non-irradiated samples. The saponins yield was the highest at a radiation dose of 6 kGy, and the detergency and bacteriostatic ability were also the strongest. After low-dose Co⁶⁰-γ irradiation, the total saponins in the S mukorossi crude extract, and detergency and bacteriostatic ability had no apparent change. Conclusion: E-beam irradiation at a dose of 6 kGy can effectively improve the content of total saponins in the crude extract of S mukorossi powder. In addition, its effects on detergency and bacteriostatic abilities are relatively significant. The findings provide sufficient reference data for the further development of S mukorossi commodities.

Keywords
Sapindus mukorossi, irradiation, bacteriostatic ability, detergency

Introduction
Sapindus mukorossi Gaertn is also known as Mu Huanzi,¹ You Huanzi, soap tree, etc. The pericarp of S mukorossi contains many saponins,² which is a type of natural non-ionic surfactant. Its extract has vigorous surface activity³ and biological activities such as bacteriostasis,⁴ oxidation resistance,⁵ and free-radical scavenging.⁶-⁸ The S mukorossi extract is an environmentally friendly washing product that microorganisms can rapidly decompose in nature without any environmental pollution.⁹

In recent years, the extraction process of S mukorossi has been gradually optimized and improved. However, the purity of the extracted products is relatively low, and bacteria multiply easily due to its rich nutrient content. Irradiation technology is a new product processing technology that can change the physical and chemical properties of materials utilizing the interactions of ionizing radiation (such as Co⁶⁰-γ ray, E-beam, x-ray) with materials, and has a significant sterilization effect.¹⁰,¹¹ It is mainly used to process perishable products such as food and cosmetics, by degrading harmful
substances and killing microorganisms, and thus, improving products’ shelf life and safety.\textsuperscript{12,13} Studies have shown\textsuperscript{14,15} that there is no radioactive residue in the irradiated products because the process only changes the molecular structure, which is harmless and non-toxic to the human body. The primary purpose of irradiation is to control microbial contamination thus delaying spoilage and improving the hygienic quality of products. Some studies have shown that irradiation can reduce the viscosity and increase the permeability of the protoplasm of plant cells, which is conducive to material extraction.\textsuperscript{16,17} Irradiation can also cause degradation of macromolecular substances leading to the cross-linking and polymerization of some compounds.\textsuperscript{12,18,19}

The purpose of this experiment is to explore how different types of irradiation (\textsuperscript{60}Co-\textgamma-ray irradiation and electron beam irradiation) affect the total saponins’ content in the crude extract of \textit{S mopokossi}. The experiment will also examine the bacteriostatic and detergency abilities of the crude extract, by treating the \textit{S mopokossi} powder with different irradiation. The study aims to acquire an effective method to improve the extraction rate of saponins from \textit{S mopokossi}, to make up for the deficiency of bacteriostatic and detergency abilities of saponins from \textit{S mopokossi}, and to provide technical support for the development of commodities of \textit{S mopokossi}.

\section*{Materials and Methods}

\subsection*{Materials}

The \textit{S mopokossi} powder (self-made in November 2017, was produced by collecting the \textit{S mopokossi} peels from the campus and then dried, crushed, and sealed; \textit{Escherichia coli}, \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, and \textit{Candida albicans} were purchased from Shanghai Lu Micro Technology Co, LTD. Commercially available bleached cotton cloth, tomato, soy sauce, lemon juice, carbon ink, snow chrysanthemum, petri dishes, glass rods, etc. were also sourced.

\subsection*{Irradiation Treatment}

E-beam radiation source: Electron accelerator (1 MeV); \gamma-ray radiation source: \textsuperscript{60}Co-\textgamma-ray radiation source

\begin{itemize}
  \item Radiation dose:
    \begin{itemize}
      \item Electron accelerator: 0, 4, 6, 8, 10, and 12 kGy.
      \item \textsuperscript{60}Co-\textgamma-ray radiation: 0, 50, 100, 150, and 200 Gy.
    \end{itemize}
\end{itemize}

The crude extract prepared from the \textit{S mopokossi} powder irradiated by the electron accelerator is labeled solution A, and the crude extract of \textit{S mopokossi} powder irradiated by \textsuperscript{60}Co-\textgamma-ray is labeled solution B.

\subsection*{Methods}

\subsubsection*{Preparation of \textit{S mopokossi} Crude Extract}

Ten grams of the \textit{S mopokossi} powder was taken and added to 90 mL distilled water, and then the mixture was heated in a microwave at a high temperature for 75 s and filtered twice. Combined, the two filtrates had a fixed volume of 270 mL. Finally, the clarified liquid was obtained using vacuum filtration and seal stored at 4°C. The treatment of each sample is the same as above.

\subsubsection*{Determination of Saponin Content}

\subsubsection*{A. Determine the Maximum Absorption Wavelength}

The total saponins content of \textit{S mopokossi} was determined using the method established by Zhou Li-bin\textsuperscript{20} (vanillin-glacial acetic acid color method). The instruments used were as follows: Shanghai ultraviolet-visible spectrophotometer UV-9000s, with oleic acid standard (purity $\geq$ 98%) as the control group, 550 nm was determined as the measurement wavelength of saponin compounds in the sample solution.
B. Draw a Standard Curve. The standard curve was drawn with absorbance A as ordinate and concentration C/(μg/ml) as the abscissa. This was repeated three times. The calculated regression equation was y = .0336x-.0048 and $R^2 = .9991$. Linear relationship was good.

C. Test the Sample. The crude extract of the saponins from the *S mukorossi* was diluted. The dilution of 100.0 μL was placed in a test tube, the rest of the process was the same as the one used to draw the standard curve. The content of total saponins in crude extract was calculated.

Determination of the Detergency of Crude Saponin Extract of *S mukorossi*

A. Prepare the Stain Solution. The fresh tomatoes and lemons, were, respectively, chopped, ground, and filtered, to obtain tomato juice and lemon juice. Boiled water was added to the extract, and the snow chrysanthemum extract was obtained.

B. Make Dirty Cloth. The appropriate size of the cloth was obtained and cut into 5 cm × 5 cm rectangle sizes. Tomato juice, lemon juice, snow chrysanthemum extract, carbon ink, and soy sauce (center, other four stains at four top corners) was added to each of the rectangular white cloth at a distance of 2 cm. The stained cloth was placed in a dryer in preparation.

C. Wash Dirty Cloth. A 100 mL *S mukorossi* diluent with a volume fraction of 3% was prepared. The three pieces of dried stained clothes were placed in glass bottles containing diluted *S mukorossi* and soaked for 12 h stirred with glass rods every 1 h. After 12 h, the stained cloth was taken out, drained, spread flat, and dried in a drying machine. The washing effect after drying was observed and scored.

D. Evaluate Detergency Ability. The detergency ability was set to 5 grades, A (no noticeable stain residue, 80-100 points), B (a little stain residue, but not noticeable, 60-80 points), C (most stain residue, 40-60 points), D (a little stain faded, 20-40 points), and E (stain basically unchanged, 0-20 points). After scoring by three to ten people, the average value was taken.

Determination of Bacterial Inhibition Zone. The bacterial inhibition zone was determined according to the method established by Zhu Chao-yang. The bacteria used were *E coli*, *S aureus*, *Bacillus subtilis*, *Candida albicans*, a total of 4 kinds of bacteria.

Bacteriostatic efficacy

$$\text{Bacteriostatic efficacy} = \frac{\text{test bacteriostatic zone diameter}}{\text{control bacteriostatic zone diameter}} \times 100\%$$

Data Analysis and Statistics

In this article, three parallel experimental data were set, and the data were statistically processed by Excel 2016 software.

Results and Analysis

Effects of Different Irradiation on Saponins Content in Crude Extract of *Sapindus mukorossi* Powder

*Sapindus mukorossi* crude extract was prepared after irradiation to test the contents for total saponins. The results are
Table 1. Inhibitory Effect of Crude Extract of Sapindus mukorossi Powder Irradiated by Electron Accelerator on Four Kinds of Bacteria.

| KGy | Bacillus subtilis | Staphylococcus aureus | Candida albicans | Escherichia coli |
|-----|------------------|----------------------|------------------|------------------|
| 0   | 33.185 ± 8.11a   | 25.350 ± 8.67a       | 24.157 ± .789a   | 21.335 ± 1.190a  |
| 4   | 38.905 ± 2.370b  | 29.873 ± 2.327b      | 28.801 ± 3.850b  | 26.406 ± 1.75b   |
| 6   | 40.623 ± 8.86b   | 31.350 ± 1.076b      | 32.149 ± .983c   | 28.167 ± .559c   |
| 8   | 35.897 ± 9.99c   | 25.926 ± 1.002a      | 25.000 ± 1.195ab | 22.481 ± 1.076a  |
| 10  | 31.867 ± .985a   | 25.311 ± .970a       | 27.461 ± .853d   | 24.643 ± .920b   |
| 12  | 24.460 ± 1.079d  | 19.137 ± .796e       | 21.466 ± 1.172a  | 21.82 ± .999a    |

Note: Different letters mean significant difference (P < .05), while the same letters mean no significant difference.

Table 2. Inhibitory Effect of Crude Extract of Sapindus mukorossi Powder After Co60-γ Irradiation on Four Kinds of Bacteria.

| KGy | Escherichia coli | Bacillus subtilis | Staphylococcus aureus | Candida albicans |
|-----|-----------------|------------------|----------------------|------------------|
| 0   | 27.121 ± 1.088a | 49.667 ± 1.73a   | 33.958 ± 1.154       | 31.852 ± 1.767a  |
| 50  | 26.667 ± 1.076  | 43.333 ± .875b   | 33.167 ± .713        | 30.167 ± .776    |
| 100 | 26.333 ± 1.238  | 46.167 ± 1.009b  | 32.667 ± .977        | 30.833 ± 1.096   |
| 150 | 27.333 ± 1.089  | 47.833 ± 1.078eb | 34.333 ± 1.018       | 32.167 ± .94     |
| 200 | 27.083 ± 1.088  | 33.167 ± .938d   | 33.667 ± .816        | 31.883 ± 1.076   |

Note: Different letters mean significant difference (P < .05), while the same letters mean no significant difference.

shown in Figure 1 and Figure 2. According to Figure 1, the total saponins content of the powder treated by E-beam was significantly different in the irradiation group and the control group. The maximum content of total saponins was .67 mg/mL at the radiation dose of 6 kGy; when the radiation dose was greater than 6 kGy, the total saponins content decreased with the increase of the radiation dose. When the irradiation dose ≤ 6 kGy, the content of total saponins in the crude extract of the S mukorossi increased, which might be due to changes in the permeability of the cytoplasmic membrane of the S mukorossi by the peroxidation reaction caused by ionizing radiation and the increase of the yield. The permeability of the cytoplasmic membrane increased with increasing radiation dose. However, the content of total saponins in crude extract of Co60-γ ray irradiated powder showed no significant change with the increase of irradiation dose. This might be due to the relatively faster repair after the cytoplasmic damage caused by low-dose irradiation. Due to storage or external conditions, the permeability of the cytoplasmic returns to its original state.16,17

The Influence of Different Irradiation on the Detergency Ability of the Crude Extract of Sapindus mukorossi Powder

Crude extracts were prepared with S mukorossi powder irradiated by electron beam and powder irradiated by Co60-γ ray. After diluted to 3% of the volume fraction, the crude extracts were used to wash self-made dirty cloth followed by scoring and statistics. Figure 3 and Figure 4 are drawn.

Both S mukorossi crude extract treated with two different irradiation treatments had a noticeable effect on the stains on the cotton cloth. Figure 3 illustrates the total detergency of A liquid increases sequentially with the increase of radiation dose. Among them, the total detergency ability of the crude extract treated with a radiation dose of 6 kGy was the highest, followed by that of the crude extract treated with 10 kGy. The total detergency ability of the crude extract of non-irradiated S mukorossi powder was the lowest. Figure 4 illustrates, after Co60-γ ray irradiation treatment, total detergency of the S mukorossi crude extract had no noticeable change. With the increase of Co60-γ ray radiation dose, the total detergency of crude extract remained constant. This is because there is no significant difference in total saponins content after irradiation at different doses, so there is no significant difference in detergency.

Effects of Different Irradiation on the Antibacterial Activity of the Crude Extract of Sapindus mukorossi Powder

Effect of Electron-Beam Irradiation on the Antibacterial Activity of the Crude Extract of S mukorossi Powder. The results from the bacteriostatic test performed on saline treatment group and crude extract groups obtained from S mukorossi powder treated with two kinds of different irradiation are shown in Table 1 and Table 2. As seen in Table 1, the antibacterial rate of solution A significantly increased, and the crude extract of the S mukorossi powder treated with 6 kGy dose had the highest antibacterial rate for different strains. With the increase of irradiation dose, the bacteriostatic rate of crude extract gradually increased, reaching the maximum at 6 kGy. According to the analysis of variance, there were differences in bacteriostasis rates in different irradiation dose groups, and there were highly significant differences between the 6-kGy irradiation dose group and the 0 kGy irradiation group. When the radiation dose exceeded 6 kGy, the bacteriostatic rate of the crude extract decreased
gradually with the increase of radiation dose. When the irradiation dose was 12 kGy, the bacteriostatic rate of crude extract was lower than that of the 0 kGy treatment group. The results showed that the high irradiation dose may reduce the bacteriostatic rate of the crude extract of the powder. Therefore, the best bacteriostatic effect can be obtained when at a radiation dose of an electron beam of 6 kGy.

**Effect of Co\textsuperscript{60-γ} Radiation on the Antibacterial Activity of the Crude Extract of** \textit{S mukorossi} **Powder.** From Table 2, it is evident that the bacteriostatic rate of the crude extract of the \textit{S mukorossi} powder treated with different doses of Co\textsuperscript{60-γ} ray irradiation did not change significantly. There was no significant difference between the 50, 100, 150, and 200 Gy irradiation group, and the 0 Gy irradiation group by ANOVA.

In conclusion, when the radiation dose of the electron beam was 6 kGy, the yield of saponins from the \textit{S mukorossi} powder was the highest, and the detergent and bacteriostatic abilities were also at the maximum. When the irradiation dose was ≤4 or ≥ 8 kGy, the extraction effect of total saponins from the powder was normal. Therefore, when the radiation dose exceeds 10 kGy, it will lead to the reduction of detergent and bacteriostatic ability of the crude extract of \textit{S mukorossi}. A low dose of Co\textsuperscript{60-γ} ray irradiation has no significant effect on \textit{S mukorossi} powder when the radiation dose is ≤200 Gy. This is because the content of total saponins, detergent and bacteriostatic ability in the crude extract of \textit{S mukorossi} were not significantly changed.

**Conclusion**

1. When the dose of Co\textsuperscript{60-γ} radiation was between 0 and 200 Gy, the extraction rate of saponins, total detergent and bacteriostasis of saponin crude extract of \textit{S mukorossi} did not change significantly compared with the control group.

2. When the electron accelerator irradiated the powder of \textit{S mukorossi}, and the irradiation dose was 0-6 KGY, the extraction rate, total detergent, and bacteriostatic power of total saponins in the crude extract of \textit{S mukorossi} increased with the increase of the irradiation dose and reached the maximum when the irradiation dose reached 6 kGy. When the irradiation dose was between 6 and 12 KGY, the extraction rate, total detergent and bacteriostasis of saponins in the crude extract of Saponins from \textit{S mukorossi} decreased with the increase of the irradiation dose.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This article is funded by Hubei Key Laboratory of Radiation Chemistry and Functional Materials Key Fund Projects (2021ZX10), and National Undergraduate Entrepreneurship Project (201910927019S).

**ORCID iD**

Yu-Min Shi https://orcid.org/0000-0003-0749-4616

**References**

1. Li S. *Compendium of Materia Medica* [M]. Beijing: China Literature and History Press; 2003:502.

2. Du M, Huang S, and Zhang J. Isolation of total saponins from *Sapindus mukorossi* gaertn. *Open J For* 2014;4(1):24-27.

3. Yang CH, Huang YC, Chang MH, Chen YF. Foam properties, detergent abilities and long-term preservative efficacy of the saponins from *Sapindus mukorossi*. *J Food Drug Anal*. 2010; 18(3):6.

4. Srinivasarao M, Lakshminarasu M, Anjum A, Ibrham M. Comparative study on phytochemical, antimicrobial and antioxidant activity of *Sapindus mukorossi* Gaertn. and Rheum emodi Wall. ex Meissn: *In vitro* studies. *Ann Phytomed*. 2015; 4(2):93-97.

5. Sushma KR, Bhavya see P, and Anitha V. Formulation of poly herbheal wash with antimicrobial activity. *Indo America J Pharma Res* 2017;7(03):7869-7872.

6. Liu D, Han eifan, and Jiang J. Microwave-assisted extraction and antioxidant activity of polysaccharides from the pericarp of *Sapindus mukorossi*. *J Forest Produc Chem Indus* 2008;38(04): 1-7.

7. Zhu C, Hu R, and Kong Q. Study on the bacteriostatic and bactericidal effect of the crude extract of *Sapindus mukorossi*. *Anhui agricultural science* 2014;4(34):12081-12086.

8. Wu H, Minping W, Xieete Y. Bioactivity and production development of saponins with *Sapindus mukorossi*. Proceedings of the 36th Annual Meeting of China Washing Products Industry, China Washing Products Industry Association; 2016:6.

9. Singh R, Kumari N, Nath G. Free radicals scavenging activity and antimicrobial potential of leaf and fruit extracts of *Sapindus mukorossi*. *Int J Phytomed*. 2016;8(1):22-28.

10. Wang L, Fang X, and Wu S. Effect of cobalt-60 irradiation on the active components of radix bupleuri. *J Pharma Anal* 2017; 37(06):1135-1141.

11. Long J, Yang L, Xiao W. Irradiation pretreatment assisted extraction of tannin from galla chinensis. *Chinese agriculture science bulletin*. 2016;32(09):188-193.

12. Xu G. *Introduction to Nuclear Agriculture*. Beijing: Atomic Energy Press; 1997:268-270.

13. Wang C, Wu X, Xu H. Application and research progress of irradiation technology in food. *Anhui Agricultural Science*. 2016;46(08):23-25.

14. Han W, Yang Z, and Xie X. Sterilization effect of irradiation on microorganisms in cosmetics and its influencing factors. *Flav Essen Cos* 2014;31(01):25-29.
15. Gong Y. To explore the application of irradiation technology in the perfume and cosmetics industry. Proceedings of the 8th Chinese Symposium on Cosmetics, 14-17 April 2011. Chinese Association of Perfume, Essence and Cosmetics Industry; 2010:55-58.

16. Vasiliev HM. Translated by Fang Yixiong etc. Effects of Ionizing Radiation on Plants. Beijing: Science Press; 1964:29-30.

17. Cui G, Shu X, and Zhang R. Study on degradation effect of wood pulp cellulose by electron beam radiation. Henan Sci 2019;37(02):179-182.

18. Li P. Effects of Irradiation on Anti-tumor Effects of Ginseng. China: Yanbian University; 2015:25-34.

19. Wang HY, SUslrN CH. Effect of Co\textsuperscript{60}-γ ray irradiation sterilization on the content of panax notoginseng R1 in panax notoginseng powder. Chinese Mod App Pharm. 2008;35(06):875-877.

20. Zhou L. Studies on Chemical Components and Surface Activity of Total Saponins from Sapindus mukorossi from Sichuan and Yunnan. Kunming, China: Yunnan University of Traditional Chinese Medicine; 2017:30-45.