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Foam Properties, Detergent Abilities and Long-term Preservative Efficacy of the Saponins from *Sapindus mukorossi*

CHAO-HSUN YANG*, YU-CHUN HUANG, YU-FEN CHEN AND MING-HSIANG CHANG

Department of Cosmetic Science, Providence University, 200, Chung-Chi Rd., Shalu, Taichung County, 43301, Taiwan. R.O.C.

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ABSTRACT

Pericarp of *Sapindus mukorossi* Gaertn has been used as a natural detergent and its extract is commercially utilized as a foam-stabilizing and emulsifying agent. The goal of this study is to investigate the foam properties, detergent ability and long-term preservative efficacy of the saponins from the pericarp of *S. mukorossi* Gaertn. The crude saponin content in the pericarp of *S. mukorossi* Gaertn was 14.2% and the total saponins content in the crude saponins extract was 85% (w/w). The foaming power of the 0.5% crude saponins extract solution from *S. mukorossi* Gaertn is 65% of 0.5% SLS solution and similar to that of 0.5% Tween 80 solution. The R5 value of 91.7% represented good foam stability of the crude saponins extracted from the pericarp of the plant. With the reduction of water surface tension from 72 mN/m to 51.7 mN/m, the 0.5% crude saponins extract solution has wetting ability. The sebum-removed experiment indicated that the crude saponins extract has moderate detergency. The results of the long-term preservative efficacy test revealed that the 0.5% crude saponins extract from the pericarp of *S. mukorossi* Gaertn is an effective preservative against *Staphylococcus aureus* ATCC 6538, but inefficacious against *Escherichia coli* ATCC 8739 and *Aspergillus niger* ATCC 16404.

Key words: *Sapindus mukorossi*, saponin, foam, detergency, long-term preservative

INTRODUCTION

Saponins are a large family of structurally-related compounds of steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provide saponins with strong surface-active property[1]. Their physiochemical and biological properties feature structural diversity which led to a number of traditional and industrial applications[2-4].

Sapindus mukorossi Gaertn (Sapindaceae) which generally grows in tropical and sub-tropical regions of Asia is a economically important agricultural plant as a source of natural surfactants. The major active ingredients of *S. mukorossi* Gaertn are saponins. Previous phytochemical studies have identified various types of saponins, including sesquiterpene oligoglycosides[5], hederagenin saponins[6-7], dammarane-type triterpenes[8] and tirucallane-type triterpenoid saponins[9-11] from the pericarp, stem and fruits of *S. mukorossi*. Pericarp of this plant has been used as a natural detergent, as well as a foam-stabilizing agent in chemical fire extinguishers. Seven kinds of saponin components including hederagenin 3-*O*-(2,4-*O*-di-acetyl-R-L-arabinopyranoside)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin 3-*O*-(3,4-*O*-di-acetyl-α-L-arabinopyranoside)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin 3-*O*-(3-*O*-acetyl-β-D-xylopyranosyl)-(1→3) -α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin 3-*O*-(4-*O*-acetyl-β-D-xylopyranosyl)-(1→3) -α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin 3-*O*-(3,4-*O*-di-acetyl-β-D-xylopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2) -α-L-arabinopyranoside, hederagenin 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, and hederagenin 3-*O*-α-L-arabinopyranoside have been isolated from the pericarp of the plant[7]. They are also added to shampoos, liquid detergents, toothpastes and beverages as emulsifier

* Author for correspondence. Tel: +886-4-26311167;
Fax: +886-4-26311167; E-mail: chyang@pu.edu.tw

and long-lasting foaming agent[12]. In addition, some pharmacological effects, such as molluscicidal[7], anti-inflammatory[13], antimicrobial[14], anthelmintic, antidermatophytic, antitussives and cytotoxic activities have been demonstrated in the saponins of plants[15].

Recent trend in food and cosmetic preservation is to avoid the use of chemical agents, leaving scientists in search of natural antimicrobial alternatives. No study has yet characterized the foam properties and detergent abilities from the saponins of *S. mukorossi*. Although foam generation has little to do with the cleansing ability of the detergent, it is an important criterion to evaluate detergent[16]. This study is thus aimed to investigate the foam properties, detergent abilities and long-term preservative efficiency of the saponins from the *S. mukorossi* Gaertn.

MATERIALS AND METHODS

I. *Chemicals*

S. mukorossi Gaertn was purchased from Dong-Xin Chemical Company (Taichung, Taiwan, R.O.C.). This sample was originally collected from the mountains of central Taiwan in Taichung County. The pericarp of the sample was washed thoroughly with tap water to remove dust and then air-dried. It was chopped and milled to 100-mesh size in a Cyclone Mill (Tecator AB, Hoganas, Sweden). Tryptone Soya Agar (TSA), Sabouraud Dextrose Agar (SDA), Modified Leethen Broth and agar were purchased from BD Diagnostic Systems (Sparks, MD, USA). Quillaja saponin, inorganic salts and all other chemicals were from Sigma (St. Louis, MO, USA).

II. *Organisms and Inocula Preparation*

The microorganisms used in this study were as follows: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538 and *Aspergillus niger* ATCC 16404. Bacteria were cultured on Tryptone Soya Agar at 37°C for 24 hours. *A. niger* was grown on Sabouraud Dextrose Agar at 25°C for 5 days.

III. *Extraction of Crude Saponins*

Saponins were extracted from *S. mukorossi* Gaertn by the method suggested by Huang[17] with slight modification. The pericarp of *S. mukorossi* Gaertn was broken into pieces, and extracted three times with boiling water. After filtration, the water extract was concentrated and partitioned once with equal volume of ethyl acetate. The ethyl acetate layer was further partitioned with equal volume of n-butanol. The n-butanol layer was evaporated, and the dry crude powder was used as crude saponins extract.

IV. *Determination of Total Saponins*

The total saponins content of extract was determined by the vanillin-sulfuric acid method[18]. This extract was mixed with vanillin (8%, w/v) and sulfuric acid (72%, w/v). The mixture was incubated at 60°C for 10 min, cooled in an ice water bath for another 15 min followed by absorbance measurement at 538 nm. Quillaja saponin was used as a reference standard[19] and the content of total saponins was expressed as Quillaja saponin equivalents (QS g/mg extract).

V. *Foaming Properties*

The method used for measuring foaming power and foam stability was developed by Ross and Miles[20]. A portion of the test solution was placed in a jacketed cylinder. Foam developed when a stream of a second portion of 200 mL test solution was added to the first portion of the test solution through a standard orifice from a 90 cm height. This resulted in turbulence and foam. The height of the foam generated was measured immediately and again after 5 min. The foam height at the initial stage indicates the foam power of the surfactant solution. The parameter R5, defined as the ratio of the height of the foam at 5 minutes to that at the initial stage, is proposed as the evaluation of foam stability[21].

VI. *Wetting Ability*

The method used to measure wetting ability was developed by Draves[22]. In the test, a five grams skein of gray cotton yarn is submerged in the test solution. The time recorded for the air in the yarn to be replaced by penetration of the solution. The end point is observed as the moment when the skein sinks. The unit of wetting ability is minute.

VII. *Surface Tension*

Surface tension of the prepared surfactants was measured at room temperature (25°C) using a Du Nouy tensionmeter (Sigma 703, KSV Instruments, USA), along with a 0.5% solution (w/v) in distilled water[23]. The surface tension of the added distilled water was 72.0 mN/m. The surfactants were aged for 30 min before further measurements.

VIII. *Detergent Ability*

The detergent ability was evaluated by a slightly modified version of the method developed by Thompson[16,24]. Hair tresses were obtained from a beauty salon. The tresses were prewashed with 5% SLS solution, dried and cut into 10 inch, 3 g swatches. The hair swatch (3 g) was suspended in 20 mL of

10% sebum solution (olive oil 20%, coconut oil 15%, stearic acid 15%, oleic acid 15%, paraffin wax 15% and cholesterol 20%) in hexane for 15 min with intermittent shaking. The swatch was removed, the solvent evaporated at room temperature and the dried hair swatch weighed to determine the sebum load. Each swatch was then split into two equal samples of 1.5 g each: one for the surfactant treatment and the other to act as an internal control to overcome the tress-to-tress variation in soil levels. The control swatch was left untreated. The test swatch was washed with 100 mL of the surfactant solution by Finger Method described by Thompson *et al*.[24]. It was then dried using a hair dryer and further dried in an oven at 60°C for 4 h to ensure uniform moisture content. The sebum remaining in the test swatch after surfactant treatment and that in the unwashed control one was then extracted using 20 mL of hexane in a stopper flask for 30 min on a rotary shaker. The hexane solution was then evaporated to dryness and the sebum extract from the test and control swatches was weighed. The detergent ability was evaluated as a percentage of sebum removed after surfactant treatment.

Detergent ability = 100 - (T × 100 /C)

T: weight of sebum in test swatch

C: weight of sebum in control swatch

IX. *Long-term Preservative Effect*

The long-term preservative test was adopted from the microbial challenge test[25]. Samples of 20 grams were placed in sterile containers and separately inoculated with bacteria and fungi suspensions to give a final level of approximate 10^6 cfu/g. After incubation periods of 0, 7, 14, 21 and 28 days at 25°C, the samples (1.0 g) were removed and placed into 9.0 mL of neutralizing medium. The plate counts on TSA or SDA were determined as the cell viability and colony-forming units after a 2-day incubation at 37°C for bacteria and a 5-day incubation at 25°C for fungi, respectively.

X. *Statistical Analysis*

All analytic measurements were taken at least in triplicate. Data were expressed as mean ± SD. Statistical comparison of means and simple correlation coefficients were conducted using the Student's *t*-test in a general linear model (GLM) procedure of an SAS system (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

The defatted powder of the pericarp of *S. mukorossi* Gaertn (15 g) was extracted with boiling water (450 mL) three times and the water extract was extracted successively with ethyl acetate and n-butanol. The percentage of crude saponins extract that was obtained from the pericarps of *S. mukorossi* Gaertn was 14.2%. The total saponins content in the crude saponins extract was 85% (w/w).

As shown in Figure 1, foam heights increased with increase in the aqueous solution concentrations. Among the three tested substances, SLS ranked the highest for the foam height of the 0.5% solutions. The foaming height of the 0.5% crude saponins extract solution from *S. mukorossi* Gaertn was 65% of 0.5% SLS solution and was similar with that of the 0.5% Tween 80 solution. These results showed that 0.5% crude saponins extract solution from the pericarp of *S. mukorossi* Gaertn possessed sufficient foam power ability. Although foam generation has little to do with the cleansing ability of the detergents, it is extremely important for the user and is therefore an important criterion in evaluating detergents[16].

Foam produced by mechanical agitation is typically an unsteady thermodynamic system. When the foam set is resting, it will decay. The rate of weakening defines the stability of the foam[26]. The change of the foam height *versus* the time is shown in Table 1. The general aspect of the curves obtained shows little difference, which reflects a good foam stability *versus* time for 0.5% SLS, Tween 80 and the crude saponins extract solutions. Foam with R5 values higher than 50% can be regarded as metastable[21]. The R5 value of 0.5% saponins solution was 91.7%, representing the good foam stability of the saponins from the pericarp of *S. mukorossi* Gaertn.

A detergent is something that increases the ability to displace air from a liquid or solid surface. The wetting phenomenon has aroused considerable commercial interest and plays a vital role in the removal of soil, dye, lubrication and printing by washing. The wetting ability of surface-active agent is commonly used to determine their comparative detergent efficacies. The Draves wetting data in Figure 2 shows that the wetting

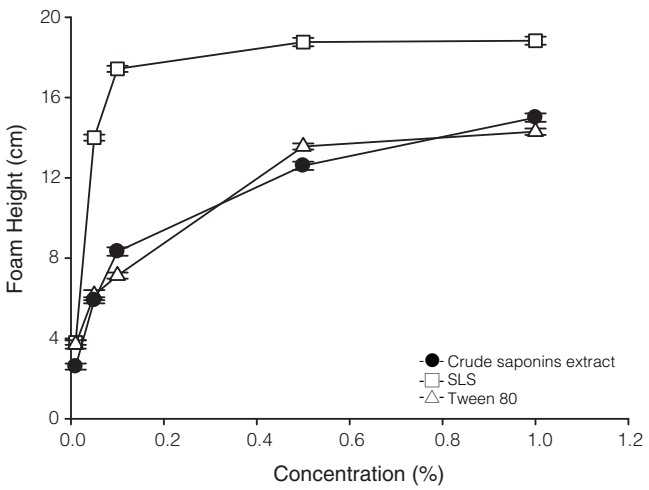

Figure 1. The foam power of various solutions.
Data are expressed as mean ± SD of three independent experiments.

Table 1. The foam heights of various solutions at the initial stage and 5 min

Solution (0.5%)	Foam height (cm)		R5*
	0 min	5 min	
SLS	18.8 ± 0.21	17.6 ± 0.19	93.6%
Crude saponins extract	12.6 ± 0.21	11.6 ± 0.18	92.0%
Tween 80	13.6 ± 0.15	13.1 ± 0.14	96.3%

*R5 is the ratio of the height of the foam at 5 min to that at the 0 min. Data are expressed as the mean ± SD of three independent experiments.

the potential to be an effective detergent. However, for commercial application, a more direct examination of detergent ability should be made. The sebum removed from 0.5% SLS, Tween 80 and crude saponins extract solutions were 90.4%, 77.6% and 60.0%, respectively (Figure 4). These results indicate SLS has excellent detergency. Crude saponins extract and Tween 80 also show moderate detergency.

The long-term preservative efficacy of the 0.5% crude saponins extract solution was tested by the method described above. As shown in Figure 5, after a 14-day incubation of *S. aureus* ATCC 6538, the bacterial number was reduced from 2.58×10^6 cfu/g to 2.50×10^2 cfu/g, and

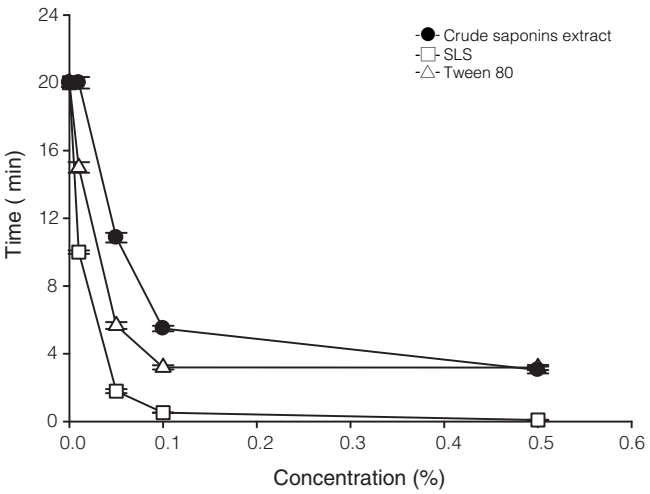

Figure 2. Variation of the wetting time *versus* the solution concentration.
Data are expressed as mean ± SD of three independent experiments.

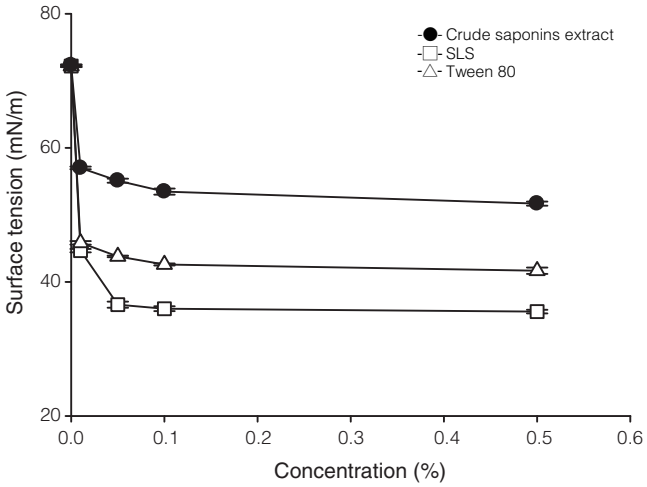

Figure 3. Variation of the surface tension *versus* the solution concentration.
Data are expressed as mean ± SD of three independent experiments.

time decreased sharply with the solution concentration ranging between 0.01-0.1%, followed by a slight drop after 0.1%. In terms of the wetting time of the 0.5% aqueous solution, the crude saponins extract solution needed 3.0 minutes to penetrate the cotton yarn while the SLS took just 0.1 minute. These data indicate that the wetting ability of the crude saponins extract of the plant was weaker than the general detergent, SLS.

Wetting phenomena are complex and depend upon several processes and factors such as diffusion, surface tension, concentration and the nature of the surface being wet. Each wetting agent has to reduce surface tension. The reduction in the surface tension of water from 72 mN/m to 32-37 mN/m with the use of commercial shampoo is considered as a good detergent[16]. As shown in Figure 3, the surface tension of 0.5% aqueous solution dropped from 72.0 mN/m to 35.6 mN/m by SLS, to 41.7 mN/m by Tween 80 and to 51.7 mN/m by crude saponins extract, respectively.

The data indicated that crude saponins extract has

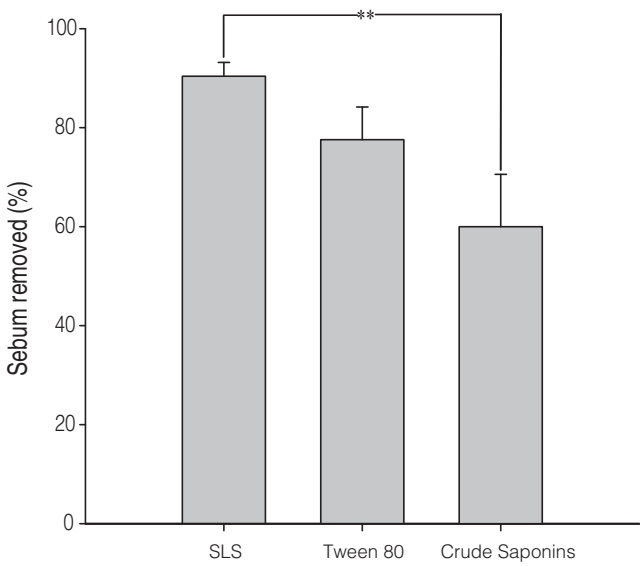

Figure 4. The percentage of sebum removed by detergents.
Data are expressed as mean ± SD of three independent experiments.
** $P < 0.01$.

further decrease took place after that. The bacterial count of *E. coli* ATCC 8739 decreased from 1.07×10^7 cfu/g to 7.02×10^4 cfu/g (14^{th} day). Slight increase (1.83×10^5 cfu/g) occurred after 21-day cultivation. The number of *A. niger* ATCC 16404 increased from 1.88×10^3 cfu/g to 9.54×10^4 cfu/g during 14-day cultivation. Based upon the USP method, an effective system of preservation would reduce the viable bacterial count to less then 0.1% of the initial concentration by the 14^{th} day. The bacterial count is measured again on the 28^{th} day and must show no increase from the Day 14 measurement. For mold, the bacterial count is measured three times, on Day 0, 14 and 28. The count did not rise above the initial level measured on Day 0[27]. These results indicated that the 0.5% crude saponins extract from the pericarp of the *S. mukorossi* Gaertn proved to be an effective preservative against *S. aureus* ATCC 6538, but ineffective against *E. coli* ATCC 8739 and *A. niger* ATCC 16404.

Previous research has shown that the saponin mixture of *Sapindus mukorossi* possesses potent antimicrobial power against dermatophytes (*Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *T. rubrum*, *Sabouraudites canis* and *Candida albicans*), no antimicrobial activity on common fungi, moderate growth inhibitory activity on Gram-positive bacteria, but no activity on Gram-negative bacteria[14]. The result obtained in this study is similar to these data.

From all these observation, it can be concluded that the saponins from *S. mukorossi* show excellent foam properties and moderate detergency. Furthermore, the results of the long-term preservative efficiency indicate that the 0.5% crude saponins extract from this plant is an effective preservative against *S. aureus* ATCC 6538, but ineffective against *E. coli* ATCC 8739 and *A. niger* ATCC 16404. These results are useful for the implementation of saponins from the *S. mukorossi* in the food and cosmetic fields.

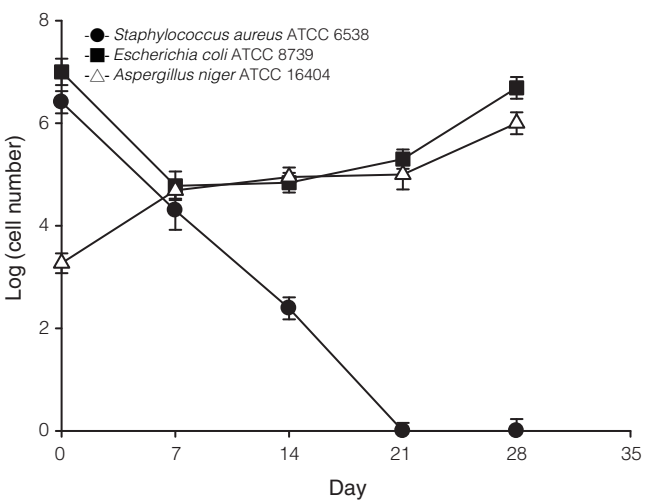

Figure 5. The viable cell numbers in the microbial challenge test. Data are expressed as mean ± SD of three independent experiments.

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