Original Article

Studying Extraction and Evaluating the Surfactant Properties of Extract from Pericarps of *Sapindus mukorossi* Gaertn. (Bo Hon) for Bio-Detergent Application

Ngoc-Nhi Nguyen Thi[1*], Minh-Chanh Nguyen[2,3], Ba-Hai Nguyen[1], Dang-Khoa Nguyen[2,3*]

[1]Binh Duong Medical College, Binh Duong 75000, Vietnam

[2]Institute of Applied Technology, Thu Dau Mot University, Binh Duong 75000, Vietnam

[3]Center of Natural Products and Food Technology, Binh Duong 75000, Vietnam

***Corresponding Authors:**

Ngoc-Nhi Nguyen Thi

E-mail: ngocnhi1979@gmail.com

Dang-Khoa Nguyen

Email: nguyendangkhoa@tdmu.edu.vn

Abstract

Saponin extract of *Sapindus mukorossi* Gaertn. (Vietnamese: Bo hon) has been a major area of interest in bio-detergent studies. In this work, we were investigating the extracting parameters of the pericarp of *S. mukorossi* and evaluating its potential as a bio-detergent. The results showed that the extraction conditions of 70% EtOH at a temperature of 80 ºC, a powder-solvent ratio of 1:10, and 180 min yielded the highest level of total saponin. The extract had a moisture content of 9.32% and a pH of 4.27–4.32, making it suitable for storage and use as a detergent. It also showed good foaming properties, with the stability of the foams maintained for more than 2 hours. Additionally, the extract did not cause dermal irritation in rabbit skin and showed inhibitory action against *Escherichia coli* at a concentration of 100 mg/mL. These findings provide more practical indications for the application of the extract of *S. mukorossi* as a bio-detergent.

Keywords: *Sapindus Mukorossi* Gaertn., Bo hon, Saponin, Bio-detergent, Extraction conditions.

1. Introduction

Surfactants are organic amphiphilic compounds that have surface active properties, some common of which are sodium dodecyl sulfate (SDS), polyoxyethylene (20) sorbitan monolaurate (Tween 20), and cetyl trimethyl ammonium bromide (CTAB) (Panda, Kumar, Mishra, & Mohapatra, 2020). Those synthetic substances are widely used in the production of soap, detergent, shampoo, and pharmaceutical industry. However, their widespread usage in households and industries poses major environmental risks due to chemical persistence in different environmental compartments such as soil, water, and sediment (Muntaha & Khan, 2015; Olkowska, Ruman, & Polkowska, 2014; Pradhan & Bhattacharyya, 2017). In addition to environmental concerns, these synthetic surfactants cause health hazards like irritation, dermatological and respiratory problems (Muntaha & Khan, 2015; Panda et al., 2020; Rowe, 2006).

The environmental and health concerns caused by synthetic surfactants have led to a proliferation of studies about natural surfactants. These surfactants have several advantages over their chemical counterparts regarding greater biodegradability, low toxicity, and biocompatibility (Rahman & Gakpe, 2008; Song, Zhu, & Zhou, 2008), which increase their possibility of applications in agriculture, cosmetics, food, and many other fields (Singh, Patil, & Rale, 2019). Corresponding to its potential, the current value of natural surfactant market is worth USD 1.2 billion and estimated to grow to 2.3 billion by 2028. The growing of the natural surfactants market is due to several contributing factors including the development of bioprocess engineering and the increasing awareness and preference of customers for natural and sustainable products (MarketsandMarkets, 2023).

In nature, surfactants can originate from bacterial fermentation or plants (Holmberg, 2001; Rai, Acharya-Siwakoti, Kafle, Devkota, & Bhattarai, 2021). Saponin are the main active

compounds in plants that possess the surfactant property. Saponin structures comprise hydrophilic and hydrophobic groups, which are oligosaccharides linked to aglycons like steroidal, triterpenoid, or alkaloidal (Sochacki & Vogt, 2022). Because of their amphiphilic structure, saponins displayed surfactant properties, forming foam in aqueous solutions; thus, they were widely applied in foods, beverages, and cosmetics. Plants high in saponins have good physicochemical and biological properties, making them a promising source of natural surfactants for scientific study and industrial use (Güçlü-Üstündağ & Mazza, 2007).

Several plants contain quite high concentrations of saponins, such as shikakai (*Acacia concinna*), soapbark (*Quillaja saponaria*), ginseng, yucca (*Yucca glauca*), and *Saponaria officinalis* (Güçlü-Üstündağ & Mazza, 2007; Rai et al., 2021). Of these, plants from the Sapindus genus–a genus of shrubs in the Sapindaceae family–have been known for their medicinal values. *S. saponaria*, which is present in America, has its fruit used by the local population for curing ulcers, external wounds, and inflammation, while *S. trifoliatus*, a native species to Asia, have been considered useful for chronic dysentery, diarrhea (Goyal, 2014). These plants are also a source of natural detergents, which have been used to wash silk and wool (Sochacki & Vogt, 2022). *S. mukorossi Gaertn.* (Vietnamese: Bo hon) is a saponin-rich plant that is widely distributed in Vietnam, China, and other Asian countries. It is traditionally used as a detergent for cleaning clothes and as shampoo for cleaning hair (Waran & Chandran, 2021; Yin, 2011). The triterpenoid saponins that are found in the plant belong to three groups, including oleanane, dammarane, and tirucallane, with high potential in environmental and cosmetic applications (Sochacki & Vogt, 2022). For instance, saponin extracted from the fruit pericarps of *S. mukorossi* was studied for its ability to wash phenanthrene from contaminated soil, with a maximum removal percentage of about 87.4% and less sorption onto soil than anion surfactant (Zhou, Wang, Chen, & Zhu, 2013). In addition, these saponins were also demonstrated to be safe for human skin, eyes, and mucous membranes (Du et al., 2015; Roy,

Kommalapati, Mandava, Valsaraj, & Constant, 1997).

The objective of this research is to study the extraction factors, including solvents, temperatures, time, and ratio extraction, that can yield the highest level of crude extracts of saponin from the pericarps of *S. mukorossi*. The obtained extracts were then tested to evaluate the potential to be applied as bio-detergent. The testing parameters were moisture, pH, foaming abilities, and foaming stability in aqueous solution. Other important characteristics including antimicrobial activity and dermal irritation were also evaluated.

2. Materials and Methods

2.1. Chemicals

Alcohol 99.5% (Xilong Chemical Co., Ltd., Guangdong, China), Luria Bertani (LB) Broth (HiMedia, India)

2.2. Plant materials

Fruit of *S. mukorossi* Gaertn. was collected in Kbang district, Gia Lai Province, Vietnam, in November, the season of harvesting fruit. Plants were identified by comparing their leaves and seeds **(Fig. 1)** to descriptions by an herbalist and botanist, Dr. Truong Thi Dep, University of Medicine and Pharmacy at Ho Chi Minh City.

2.3. Investigating the extraction

The collected fruits were dried at 60 ºC until the residual moisture was below 10%. The pericarps were separated from the fruits and pulverized through a sieve with a mesh size of 0.5 mm.

Solvents

The obtained powders were immersed in different solvents: water, 70%, and 90% EtOH. The power was repeatedly extracted at 100 ºC. The solid was weighed to determine which solvent yielded the highest total crude extract.

Temperatures

Dried powders were immersed in 70% EtOH and extracted at four different temperatures: 70, 80, 90, and 100 ºC. Like the investigation of solvents, the temperature that yielded the highest amount of extract was applied for the next screening experiment.

Time of extraction

Grinned powders were put into 70% EtOH and extracted at 80 ºC for five periods: 90, 120,

150, 180, and 210 min. The suitable time was the period that yielded the highest amount of crude extract.

Powder-solvent ratio

Powder and solvents were extracted at different ratios of 5:1, 10:1, and 15:1. The extraction was conducted using 70% alcohol at 80 ºC for 180 min, and the amount of extract was measured to determine the suitable ratio of powder and solvent.

2.4. Phytochemical screening

The phytochemical screening tests of the different saponin extracts were conducted according to Vietnamese Herbal Pharmacopoeias and previous studies (Hieu et al., 2023; Van-Anh et al., 2021): flavonoids test with cyanidin, triterpenoid test with Liebermann-Burchard reagent, cardiac glycoside test with Raymond-Marthoud and Xanthydrol reagents, anthraquinone and coumarin test with 10% NaOH, saponins test by foam test, tannin test with 5% $FeCl_3$, and alkaloid test with Mayer and Bouchardat Dragendorff reagents.

2.5. Physicochemical properties of the extract

Moisture

According to the Pharmacopoeia standard, the moisture in the extract was kept below 20%. The evaporation occurred in a water bath at a temperature under 80 ºC. The water content was then measured with an infrared moisture analyzer (MA100Q, Sartorius Group, Germany).

pH

Determination of pH using the potentiometric method followed by the National Standard for bio-surfactants (The Directorate of Standards, 2018), which was based on ISO-4316:1977. The total extract (20 g) was weighed and diluted with distilled water (5-20% w/w), and then the pH range of different concentrated solutions was measured using a pH meter (EBRO PHT, Ingolstadt, Germany).

2.6. Evaluation of foaming activities

Foam high

The extract was added into ten test tubes (16 x 160 mm) with a corresponding volume of 1–10 mL, followed by the supplementation of distilled water to reach a final volume of 10 mL. Each test tube was vertically shaken for 15 seconds and left to standstill for the next 15 minutes. The height of the foam columns was measured in cm (Tmáková, Sekretár, & Schmidt, 2016).

Foam stability

The extracts and standards (commercial detergent) were diluted by 2-time distilled water to a concentration of 10% (w/w) and pH 6.7. Each solution was stirred using a magnetic stirrer (IKA C-Mag HS 4, Staufen, Germany) with a 3-cm magnetic bar for 5 min. Then the height of the bubble column was measured by a scale, and the durability of the bubble was also compared after 2 h (Tmáková et al., 2016).

2.7. Antimicrobial activity

The antibacterial activity of the extract was tested against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 25922) by the agar-well diffusion method (Lehrer, Rosenman, Harwig, Jackson, & Eisenhauer, 1991). *E. coli* was cultivated in LB medium overnight, then diluted to a density of 10^6 CFU/mL (compared with standard McFarland 0.5 turbidity tubes). Bacterial broth (0.1 mL) was then evenly spread onto the agar surface. Use a specialized rod to make holes 5 mm in diameter on the surface of the agar that was covered with bacteria. Extracts (50 µL) were put into the agar hole and incubated at 35 ºC for 24 hours. After incubation, the antibacterial ring radiuses were measured according to the equation.

$$R = D - d \text{ (mm)}$$

(In which, R: antibacterial ring radius; D: diameter of antibacterial ring measured; d: diameter of a 5 mm agar hole).

2.8. Dermal irritation test

The animal handling approaches pertain to national regulations of ethics on the care and use of animals. The permission numbers 435/QĐ-CĐYT and 312/QĐ-CĐYT were approved by the Ethical Review Board of Binh Duong Medical College.Two-month-old white New Zealand rabbits (n = 3) were housed in individual cages in a controlled environment with a maintained temperature of 25 ºC and a relative humidity of 70%. The dermal irritation of the extract was tested using the patch test method (Du et al., 2015). Rabbit ears and backs were shaved, cleaned with distilled water, and antisepticized with 70% ethanol. Extracts (0–100 mg/mL) were spread on the test region and covered by patches. After 48 hours, the test regions on the skin were checked and photographed. The results were negative when there was no lesion or change in skin color at the application site, a weak positive reaction when pink or red patches appeared on the test skin, and a strong positive reaction when blisters or sores appeared.

2.9. Statistical analysis

All experimental values were presented as the means ± SD of three independent experiments. A one-way analysis of variance was used to make the statistical comparison using Graph Pad Prism (8.4.2, Graph Pad Software, San Diego, California, USA). Statistical comparison was performed by one-way analysis of variance by Graph Pad Prism (8.4.2, Graph Pad Software, San Diego, California, USA). A p value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. The extraction of *Sapindus mukorossi* Gaertn.

We investigated the effects of water and alcohol on the yield of extraction. The results showed that water and 70% EtOH produced the highest amount of extract, roughly 90% (w/w), higher than the yield of 90% EtOH, approximately 85% ($p < 0.05$) **(Fig. 2A)**. Although water and 70% EtOH resulted in similar amounts of crude extract, 70% EtOH sped up the condensation process, so 70% EtOH was chosen for the next investigations. The temperatures that yielded the highest amount of crude extract were 80 ºC and 90 ºC **(Fig. 2B).** For power efficiency, 80 ºC was chosen as the suitable temperature to extract total saponin. Similarly, the extraction periods that produced the highest crude extract, approximately 73–74%, were 180–210 min (**Fig. 2C**). Thus, 180 minutes was determined to be the optimal period when considering the economic factor. For the powder-solvent ratio, the highest yield of total extract was obtained at the ratios of 1:10 and 1:15 (w/v) **(Fig. 2D).** The ratio of 1:10 was found to be the optimal ratio for subsequent experiments.

3.2. Phytochemical screening

The result of the phytochemical screening of water and 70% EtOH extracts of *S. mukorossi* is displayed in Table 1. The results revealed the absence of triterpenoid, cardiac glycoside, anthraquinone, coumarin, and alkaloids. In contrast, these extracts contain flavonoids, tannins, and saponins.

3.3. Physicochemical properties

The moisture and pH of the extract are shown in Table 2. The moisture was 9.32%, which is smaller than the standard of 20% and thus meets pharmacopeia standards. The pH values of the 5-20% extract solutions were in the range of 4.27–4.32, indicating the weak acidity of crude

S. mukorossi extract. These results were consistent with previous studies, showing that the extract of *S. mukorossi* was weakly acidic. The pH ranges of *S. mukorossi* extract were reported to be 4.5–4.72 at concentrations of 0.1-0.4% (Tmáková et al., 2016) or 5–5.8 at concentrations of 0.01–1% (Zhou et al., 2013). The hydrophilic end of saponin molecules was shown to contain glucuronic acid, which causes this weak acidity (Mitra & Dungan, 1997).

3.4. Foaming activities

Mechanical agitation was used to create foam in the aqueous solution of the extract. The results showed that foam was formed at all investigated concentrations, with the height ranging from 2.8 to 4.9 cm **(Fig. 3)**, while distilled water (the negative control) did not generate foam. This indicates the presence of saponin in the crude extract of the studied *S. mukorossi*. In addition, the increase in the height of the foam corresponded with the rising concentration ($R^2 = 0.8899$). This result accorded with earlier studies, demonstrating the positive correlation between foam height and saponin concentration in aqueous solutions (Chen, 2010; Hajimohammadi, Hosseini, Amani, & Najafpour, 2016).

The extract solution also generated higher foam than the commercial detergent solution at a similar concentration **(Fig. 4A),** with corresponding average heights of 11.58 and 9.04 cm **(Table 3)**. This outcome confirmed the excellent foaming properties of *S. mukorossi* extract (Yang, 2010). Tmáková *et al.* (2016) also showed that the extract of *S. mukorossi* generated substantially higher foam than tween 80 ($p < 0.05$), a nonionic surfactant (Tmáková et al., 2016). Furthermore, we investigated the stability of the foam. Due to their unstable thermodynamic properties, foams started to decompose when mechanical agitation was stopped, and the system was left to rest. The foam heights of commercial detergent were much less stable than those of *S. mukorossi* extract, as its height decreased to 4.25 cm while that of *S. mukorossi* extract was maintained at 10.86 cm (**Fig. 4 and Table 3**). The top-down

observation showed the corresponding outcome: fewer foams were observed in commercial detergent than in *S. mukorossi* extract **(Fig. 4).** The stability of the foam of *S. mukorossi* extract may be attributed to its surfactant activity. Another study suggests that the soapnut solution resulted in decreased surface tension and increased density and viscosity, thus causing foam stability (Panda et al., 2020).

3.5. Antimicrobial activity

The antibacterial activity of the extract against *E. coli* was evaluated using agar-well diffusion to determine its potential as a bio-detergent. The extract of *S. mukorossi* showed inhibitory activity on *E. coli* at a concentration of 100 mg/mL, with a clear inhibition zone appearing at this concentration **(Fig. 5)**. The antibacterial ring radius was 15.32 ± 0.04 mm. This result provides more antibacterial evidence for *S. mukorossi's* extract. A previous study also showed antibacterial activity of *S. saponin* against various bacterial strains: *Salmonella paratyphi* A (CMCC 50095), *Shigella dysenteriae* (CMCC 51334), *Listeria welshimeri* (ATCC 35897), *Escherichia coli* (ATCC 8099), *Pseudomonas aeruginosa* (ATCC 15442), and *Staphylococcus aureus* (ATCC 6538) (Heng, 2014).

3.6. Dermal irritation

Skin irritation is one important criterion for a safe biodetergent, so we examined this index using a patched test assay. The result showed that the extract of *S. mukorossi* did not cause dermal irritation or redness at a concentration of 0-100 mg/mL **(Fig. 6)**. This result accords with previous studies showing that external use of saponins was safe for human skin, eyes, and mucous membranes (Du et al., 2015; Pradhan & Bhattacharyya, 2017; Roy et al., 1997).

4. Conclusion

This study successfully obtained the crude saponin extract of *S. mukorossi* that has the potential

to be used as a bio-detergent. The optimal conditions —70% EtOH, a temperature of 80 ºC, a powder-solvent ratio of 1:10, and 180 min—yielded the highest level of crude extract. The overall quality of the extract met the standard for the development of bio-detergents, with a moisture content of 9.32% and a pH remaining at 4.27–4.32. The extract displayed good foaming activities, forming foams at all given concentrations and stabilizing foams more than commercial detergent. In addition, the extract showed inhibitory activity against *E. coli* at a concentration of 100 mg/mL and did not induce any dermal irritation on rabbit skin. Future applied studies should focus on developing formulas and testing the usage of products from this extract to further support the use of bio-detergents.

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Declaration of interests

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

Author contributions

Ngoc-Nhi Nguyen Thi: Investigation, Formal analysis, Conceptualization, Methodology; Minh-Chanh Nguyen: Investigation, Formal analysis, Conceptualization, Methodology; Ba-Hai Nguyen: Conceptualization, Methodology; Dang-Khoa Nguyen: Formal analysis, writing – Original Draft, Writing - Review & Editing

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Tables

Table 1. Phytochemical Screening of Extracts of *S. mukorossi*

Phytochemical compounds	Testing methods	Extracts	
		Aqueous extract	EtOH extract
Triterpenoid	Liebermann–Burchard	-	-
Cardiac glycoside	Raymond–Marthoud Xanthydrol	-	-
Anthraquinone	Sodium hydroxide	-	-
Coumarin	Sodium hydroxide	-	-
Flavonoid	Cyanidin	+	+
Saponin	Foam test	+	+
Tannin	5% $FeCl_3$	+	+
Alkaloid	Mayer Bouchardat Dragendorff	-	-

Presence (+); Absent (-)

Table 2. Moisture and pH values of plant extracts at different concentrations

Moisture	pH	
	Concentration (%)	pH
9.32 ± 0.29	5	4.32 ± 0.02
	10	4.29 ± 0.00
	20	4.27 ± 0.01

Figure 1. *Sapindus mukorossi* Gaertn. (A) Leaves; (B) Raw fruits; (C) Ripe fruits; (D) fruits and seeds

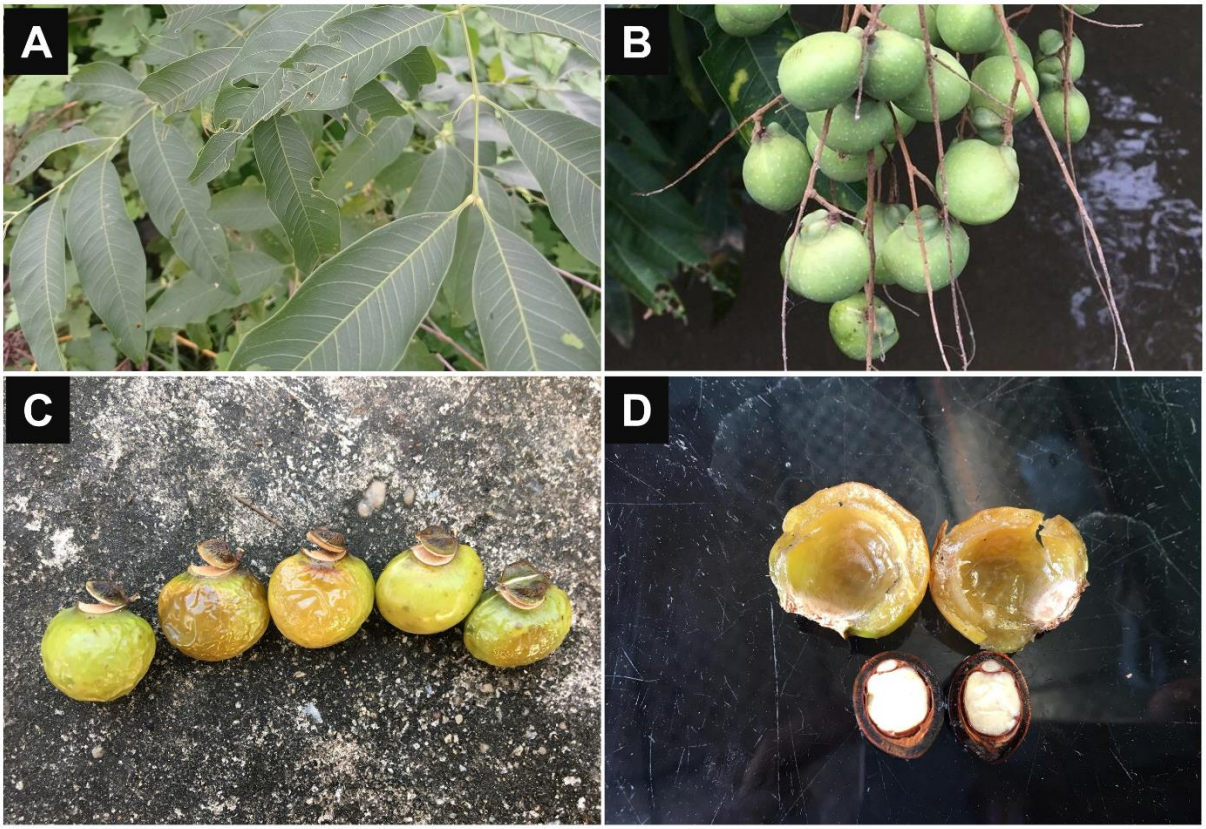

Figure 2. Efficient extraction of *Sapindus mukorossi* Gaertn. according to different factors. (A) Solvents; (B) Temperature; (C) Time; (D) Powder-solvent ratio; Letters a, b, and c, indicate the statistically significant difference among values ($p < 0.05$).

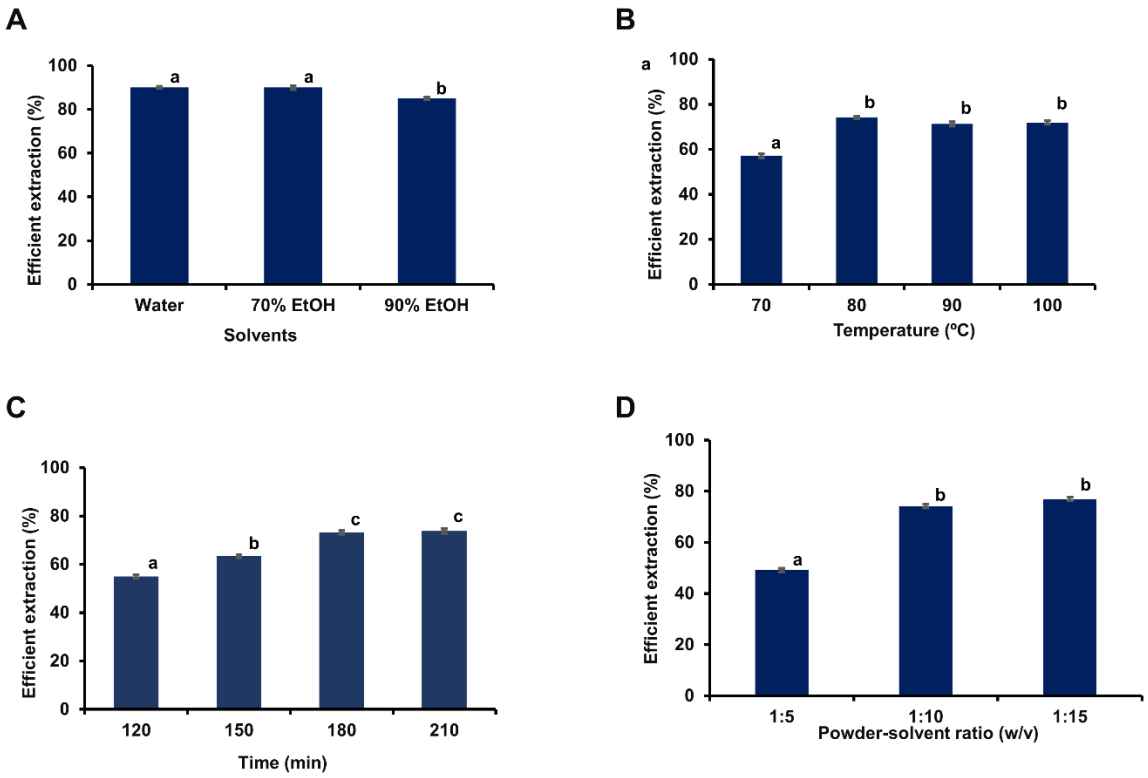

Figure 3. Foam height according to different concentrations

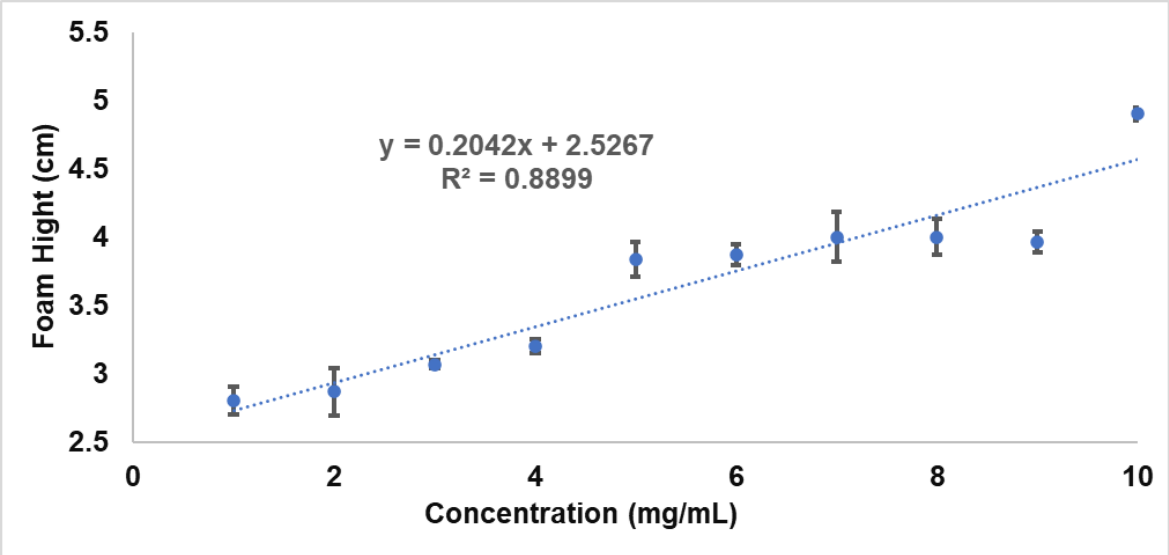

Figure 4. Comparison of foam height and foam stability of *Sapindus mukorossi* Gaertn. extract and commercial detergent. (A): 0 h, (B): 2 h (top-down view on the left).

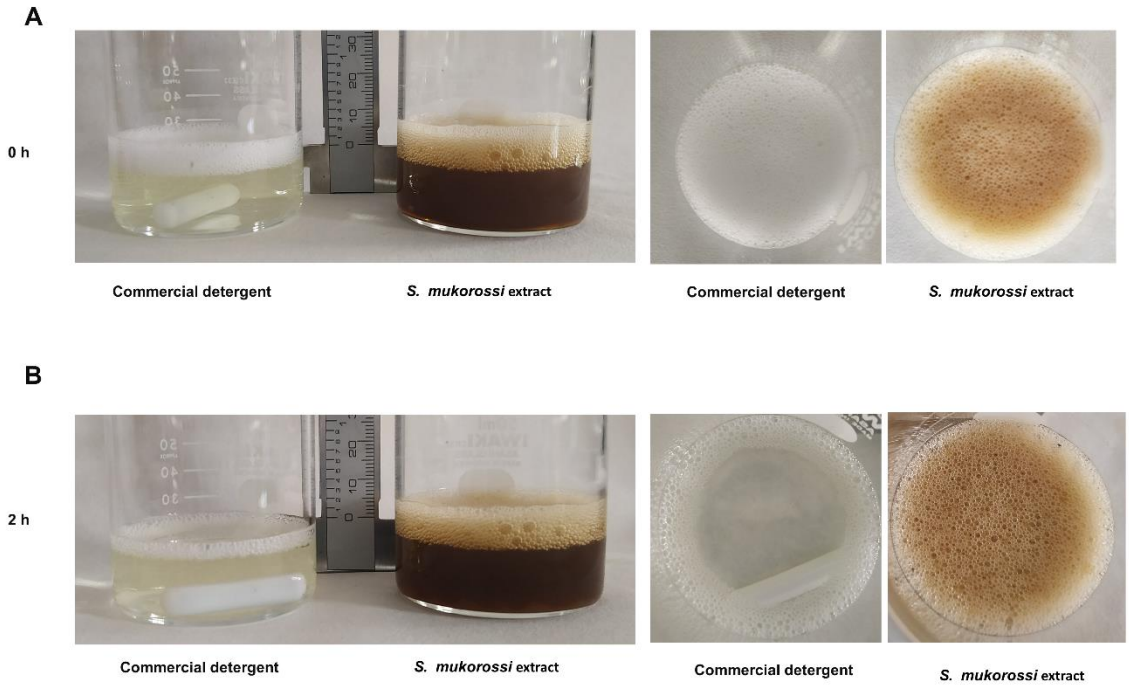

Figure 5. Antimicrobial activity against *Escherichia coli* at concentrations of (A) 25 mg/mL; (B) 50 mg/mL; (C) 100 mg/mL, NC: negative control

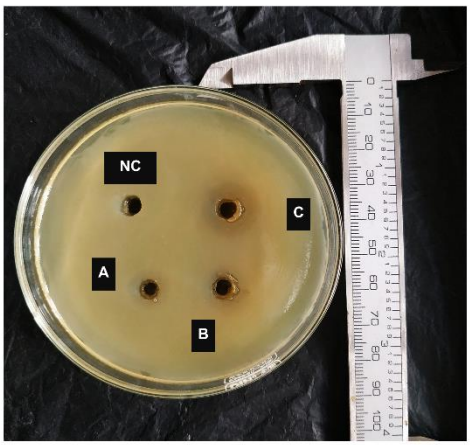

Figure 6. Dermal irritation test. (A) White New Zealand rabbits; (B) Extract solution (0-100 mg/mL); (C) 100 mL was infused in each patch; (D) The back of white rabbits was shaved; (E) Patches were adhesive to the skin; (F) Skin after 48.