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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], and Dang-Khoa Nguyen[2, 3*]

[1] *Binh Duong Medical College, Thu Dau Mot, Binh Duong, 75000 Vietnam*

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

[3] *Center of Natural Products and Food Technology, Thu Dau Mot, Binh Duong, 75000 Vietnam*

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Abstract

Saponin extract of *Sapindus mukorossi* Gaertn. (Vietnamese: Bo hon) has been of major interest in bio-detergent studies. In this work, we investigated the extraction parameters of the pericarp of *S. mukorossi* and evaluated potential of the extract as a bio-detergent. The results showed that extraction with 70% EtOH at a temperature of 80 ºC, a powder to solvent ratio of 1:10, and 180 min duration, 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 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 ones being 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, and shampoo, as well as in the pharmaceutical industry. However, their widespread use in households and industries poses major environmental risks due to chemical persistence in various 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 by irritation, including dermatological and respiratory problems (Muntaha & Khan, 2015; Panda *et al.*, 2020; Rowe, 2006).

The environmental and health concerns associated with synthetic surfactants have led to a proliferation of studies about natural surfactants that have several advantages over their chemical counterparts regarding greater biodegradability, low toxicity, and biocompatibility (Rahman & Gakpe, 2008; Song, Zhu, & Zhou, 2008), which increase their potential for applications in agriculture, cosmetics, food, and many other fields (Singh, Patil, & Rale, 2019). Corresponding to such potential, the current value of natural surfactant market is about USD 1.2 billion and is estimated to grow to 2.3 billion

*Corresponding author
Email address: nguyendangkhoa@tdmu.edu.vn

by 2028. The growth in 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 the nature, surfactants can originate from bacterial fermentation or plants (Holmberg, 2001; Rai, Acharya-Siwakoti, Kafle, Devkota, & Bhattarai, 2021). Saponins are the main active compounds in plants that possess surfactant properties. Saponin structures comprise hydrophilic and hydrophobic groups, which are oligosaccharides linked to aglycons of steroidal, triterpenoid, or alkaloidal types (Sochacki & Vogt, 2022). Because of their amphiphilic structure, saponins display surfactant properties, forming foam in an aqueous solution; thus, they are 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). Among these, plants from the Sapindus genus – a genus of shrubs in the Sapindaceae family – have been known for their medicinal value. *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, has been considered useful for chronic dysentery or 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 the three groups 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 an 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 was to study the extraction factors, including choice of solvent, temperature, time, and proportions in extraction, to maximize the level of crude saponin extract from the pericarps of *S. mukorossi*. The obtained extracts were then tested to evaluate the potential for serving as a bio-detergent. The tested parameters were moisture, pH, foaming ability, and foam stability in an 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), and Luria Bertani (LB) Broth (HiMedia, India)

2.2 Plant materials

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

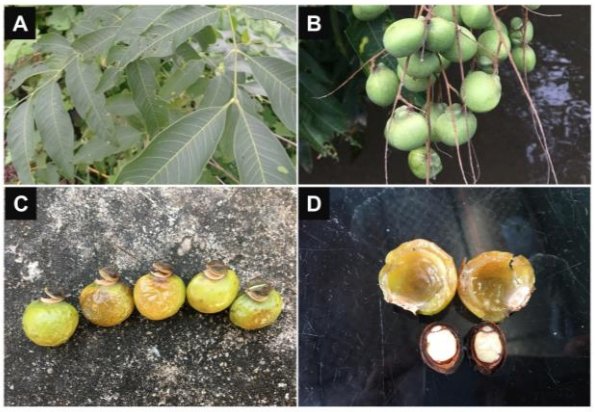

Figure 1. *Sapindus mukorossi* Gaertn. (A) Leaves, (B) raw fruit, (C) ripe fruit, and (D) fruit and seed

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 fruit and pulverized through a sieve with a mesh size of 0.5 mm.

2.3.1 Solvent

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

2.3.2 Temperature

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

2.3.3 Time of extraction

Ground powders were put into 70% EtOH and extracted at 80 ºC for the five durations 90, 120, 150, 180, and 210 min. The suitable time duration was that which yielded the highest amount of crude extract.

2.3.4 Powder-solvent ratio

Powder to solvent ratios 5:1, 10:1, and 15:1 were tested for extraction with 70% alcohol at 80 ºC for 180 min, and the amount of extract was measured to determine the most 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 were tested with cyanidin, triterpenoids were tested with Liebermann-Burchard reagent, cardiac glycosides were tested with Raymond-Marthoud and Xanthydrol reagents, anthraquinone and coumarin were tested with 10% NaOH, saponins were assessed by foam test, tannins were tested with 5% $FeCl_3$, and alkaloids were tested with Mayer and Bouchardat Dragendorff reagents.

2.5 Physicochemical properties of the extract

2.5.1 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).

2.5.2 pH

Determination of pH using the potentiometric method followed 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 activity

2.6.1 Foam height

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

2.6.2 Foam stability

The extracts and standards (commercial detergent) were diluted by twice 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 bubbles 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. A special rod was used to make 5 mm diameter holes on the surface of the agar that was covered with bacteria. Extracts (50 µL) were put into the agar holes 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 is antibacterial ring radius, D is measured diameter of the antibacterial ring, and d is 5 mm as diameter of the hole.

2.8 Dermal irritation test

The animal handling approaches conformed 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 was recorded 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 are presented as mean ± SD of three independent experiments. A one-way analysis of variance was used for statistical comparisons in 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 with 90% EtOH that was approximately 85% ($p < 0.05$) (Figure 2A). Although water and 70% EtOH resulted in similar amounts of crude extract, 70% EtOH sped up the condensation process, so this was chosen for the next investigations. The temperatures that yielded the highest amount of crude extract were 80 ºC and 90 ºC (Figure 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 (Figure 2C). Thus, 180 minutes was determined to be the optimal period when considering economic aspects. Regarding the powder-solvent ratio, the highest yield of total extract was obtained at the ratios of 1:10 and 1:15 (w/v) (Figure 2D). The ratio 1:10 was considered optimal for the subsequent experiments.

3.2 Phytochemical screening

The results from phytochemical screening of water and 70% EtOH extracts of *S. mukorossi* are displayed in Table 1. They reveal the absence of triterpenoids, cardiac glycosides, 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 20% maximum allowed, and thus meets the pharmacopeia standards. The pH of the 5-20% extract solutions was in the range 4.27–4.32, indicating weak acidity of the crude *S. mukorossi* extract. These results are consistent with previous studies, showing that the extract of *S. mukorossi* is weakly acidic. The pH range of *S. mukorossi* extracts has been 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 (Figure 3), while distilled water (the negative control) did not generate foam. This indicates the presence of saponins in the crude extract of the studied *S. mukorossi*. In addition, height of the foam increased with the

Table 1. Phytochemical screening of extracts of *S. Mukorossi*

Phytochemical compounds	Testing method	Extracts	
		Aqueous extract	EtOH extract
Triterpenoids	Liebermann–Burchard	-	-
Cardiac glycosides	Raymond–Marthoud	-	-
	Xanthydrol		
Anthraquinone	Sodium hydroxide	-	-
Coumarin	Sodium hydroxide	-	-
Flavonoids	Cyanidin	+	+
Saponins	Foam test	+	+
Tannins	5% $FeCl_3$	+	+
Alkaloids	Mayer	-	-
	Bouchardat		
	Dragendorff		

Present (+); Absent (-)

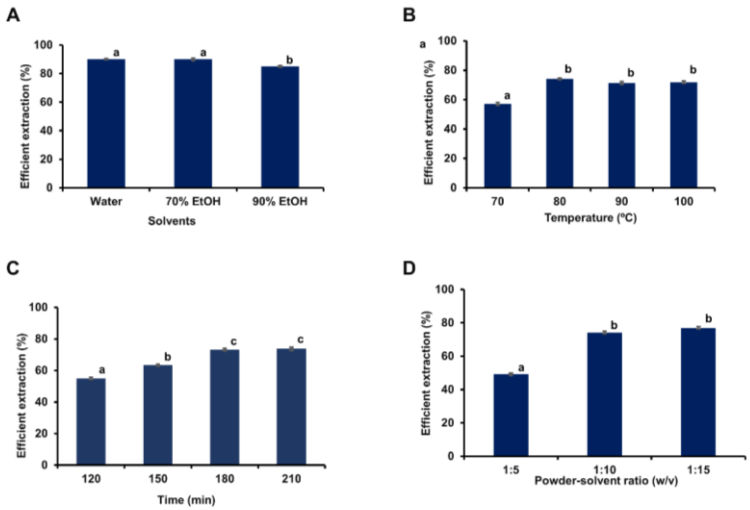

Figure 2. Testing choices in extraction of Sapindus mukorossi Gaertn. for (A) solvent, (B) temperature, (C) time, and (D) powder to solvent ratio. Letters a, b, and c indicate statistically significant differences among the values ($p < 0.05$).

Table 2. Moisture of plant extract and pH at various aqueous concentrations

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

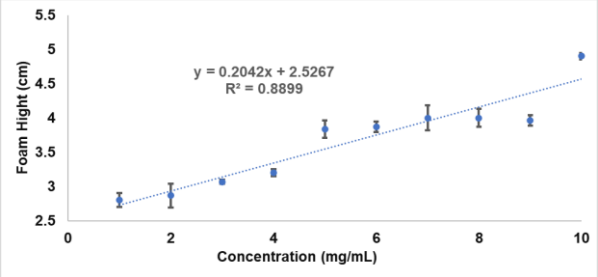

Figure 3. Foam height by aqueous concentration

concentration (R^2 = 0.8899). This agrees with earlier studies, demonstrating 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 (Figure 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 height of commercial detergent was much less stable than that of *S. mukorossi* extract, as its height decreased to 4.25 cm while that of *S. mukorossi* extract was maintained at 10.86 cm (Figure 4 and Table 3). The top-down observation showed the corresponding outcome: less foam was observed for the commercial detergent than for the *S. mukorossi* extract (Figure 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, which contributed to foam stability (Panda *et al.*, 2020).

Table 3. The foam heights of crude saponin extract and commercial detergent initially and after resting for 2 h

Solution (10%)	Foam height (cm)	
	Initial	After 2 h
Crude saponin extract	11.58 ± 0.59	10.86 ± 0.35
Commercial detergent	9.04 ± 0.49	4.25 ± 0.27

3.5 Antimicrobial activity

The antibacterial activity of the extract against *E. coli* was evaluated using agar-well diffusion to determine potential of the extract 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 (Figure 5). The antibacterial ring radius was 15.32 ± 0.04 mm. This evidences the antibacterial activity of *S. mukorossi* 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

Absence of skin irritation is an important criterion for a safe biodetergent, so we examined this aspect using a patch test assay. The results show that the extract of *S. mukorossi* did not cause dermal irritation or redness at a

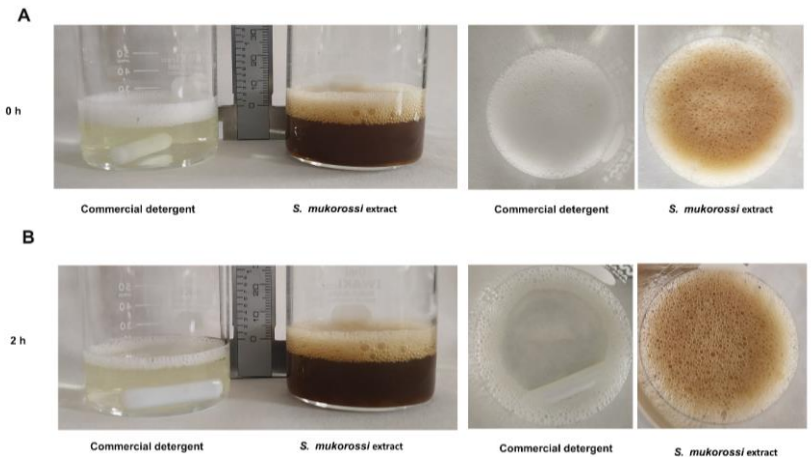

Figure 4. Comparison of foam height and foam stability of *Sapindus mukorossi* Gaertn. extract with a commercial detergent. (A) at 0 h, and (B) at 2 h (top-down view on the right).

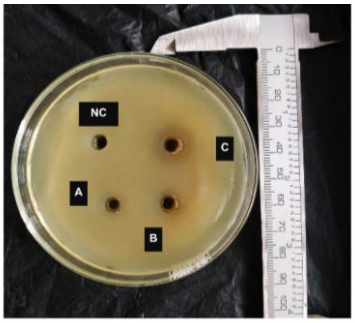

Figure 5. Antimicrobial activity against Escherichia coli at concentrations of (A) 25 mg/mL, (B) 50 mg/mL, and (C) 100 mg/mL. NC stands for negative control

concentration of 0-100 mg/mL (Figure 6). This agrees with previous studies showing that external use of saponins is safe on human skin, eyes, and mucous membranes (Du *et al.*, 2015; Pradhan & Bhattacharyya, 2017; Roy *et al.*, 1997).

4. Conclusions

This study successfully obtained the crude saponin extract of *S. mukorossi* that has potential for use 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 within 4.27–4.32. The extract displayed good foaming activity, forming foams at all given concentrations, with foam stability superior to a 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 formulations from this extract and testing their use in products to further support the use of bio-detergents.

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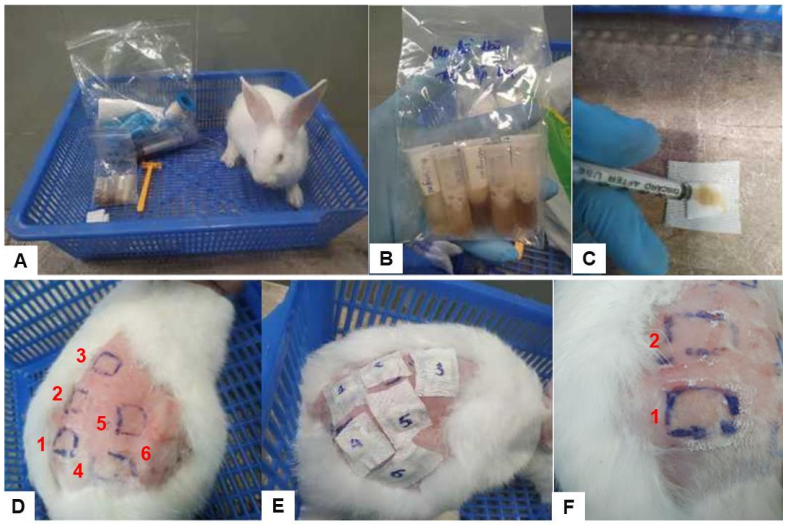

Figure 6. Dermal irritation test. (A) White New Zealand rabbits, (B) extract solution (0-100 mg/mL), (C) 100 mL was infused in each patch, (D) the backs of white rabbits were shaved, (E) patches were attached to the skin; and (F) skin after 48 hours.

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