Improvement of Detergency Properties and Anti-Microbial Action of Biosoaps Using Saponin-Rich Extract and A-Bisabolol Essential Oil

Nguyen Van-Anh, Nguyen Thi Thom, Hoang Huu Hiep, Cao Hong Ha^{*}

School of Chemical Engineering, Hanoi University of Science and Technology, Ha Noi, Vietnam *Corresponding author email: ha.caohong@hust.edu.vn

Abstract

In this study, natural ingredients were added to bio-soaps, synthesized from castor oil, a plant-derived ingredient, to improve the detergency, anti-bacterial, and stability capabilities (saponin from Soapacean fruit and essential oil of chamomile) of such soap. The saponin from soapnut fruit was extracted by an extraction process based on ethanol extraction. The saponin concentration in the extract was determined by the UV-Vis spectroscopy method. By evaluating the height and stability of the foam column, the surface tension using the Du Noüy method, and critical micelle concentrations (CMC), we looked into the efficiency of saponin in enhancing soap properties. The results show that saponin will reduce the value of CMC and, at the same time, expand the working limit of soap in brackish water and hard water (containing Mg²⁺ and Ca²⁺ ions). In addition, the presence of saponins and α -Bisabolol essential oil enhanced the anti-bacterial properties of the soap. These results expand the possibility of using natural substances in making green products that are clean and safe for humans and the environment.

Keywords: α-Bisabolol essential oil, anti-bacterial properties, bio soaps, saponins

1. Introduction

Surfactants play the most crucial role in formulating cosmetics and hygiene products because they are responsible for forming emulsions, foams, and cleaning activities [1]. However, most commercial surfactants are manufactured from crude oil, a nonrenewable source. Moreover, they may be harmful to the ecology because of their difficulty in degradation when discharged into the environment. Therefore, the development of biosurfactants (e.g., bio soaps or/and surfactants extracted from plants) to alter the chemically synthetic substances has attracted much attention due to their advantages such as environmental friendliness, natural degradation, nontoxicity to humans, and good cleaning ability [2].

Bio-soaps (biological soaps) are biosurfactants derived from natural and renewable materials (such as vegetable oils or animal fat). Their excellent properties, such as renewable ability, easiness in preparation. biodegradation under and mild conditions, enable them to be potential surfactant candidates [3]. However, the nature of bio-soaps is the alkaline salts of fatty acids (anionic surfactants), so their solubility is poor in the presence of positivecharged ions in hard water or brackish water, resulting in their low activity [4]. Hence, it is necessary to add auxiliary substances to improve the properties of biosoaps so they can be used in severe environments. Therefore, the direction of most interest is the addition of non-ionic surfactants derived from nature, such as

ISSN 2734-9381

https://doi.org/10.51316/jst.164.etsd.2023.33.1.2

saponin group compounds (Fig. 1). In addition, the non-anionic surfactant is known that they can work well in hard water or brackish water.



Fig. 1. The structure of saponin extracted from soapnut (Sapindus Mukorossi)

Among the saponin family, compounds extracted from the pericarp of soapnut (Sapindus Mukorossi) are often used as raw materials for the industry of hand protection products, medicines, industrial additives, and washing powders due to their anti-dandruff, antiitch, and anti-inflammatory activity, anti-bacterial properties anti-fungal and emulsifying ability and good foaming [5].

M. D. Köse *et al.* [6] carried out the extraction of Saponins from Soapnut by using some different solvents and showed that the concentration of saponin

Received: October 9, 2022; accepted: November 15, 2022

ranged between 19-30 wt.%. This saponin amount in the raw materials was higher than the average value of saponin shown in the literature review.

A-Bisabolol is an essential oil containing monocyclic sesquiterpene alcohol that was first and extracted identified from Matricaria chamomilla [7]. It has been used commonly as a fragrance ingredient in cosmetics and personal care product formulations due to its significant biological properties such as anti-inflammatory, anti-irritant, anti-inflammatory, anti-microbial, and anti-fungal activities [8]. In addition, α -bisabolol is used in the formulation of a wide variety of products, including facial makeup, skincare, and hair care products. Moreover, its safety and low toxicity [9] are why it is often chosen, and the presence of even a small amount of α -bisabolol (proposed < 1 wt.%) may have a significant effect.

In this study, we reported the strategies to enhance the properties of nature-derived bio-soaps by adding saponins extracted from soapnuts pericarp and α -Bisabolol essential oil. The saponin compound could widen the bio-soaps working range in severe environments, whereas α -Bisabolol could improve its anti-microbial properties.

2. Experiments

2.1. Materials

Saponin was extracted from soapnuts purchased from Đắk Lắk, Vietnam. Ethanol (EtOH 99,5%), H₂SO₄ (98%), vanillin, KOH, CaCl₂, and NaCl at analytical grade were purchased from China. Saponin (20 – 35 wt.%) was purchased from Sigma-Aldrich (CAS. 8047-15-2). Sodium linear alkyl benzene sulfonate (LAS) was purchased from VietChem Company, Vietnam. Chlorhexidine gluconate (CHG) was purchased from India. Essential oil containing 45 wt.% α -bisabolol was supplied by VietChem Company, Vietnam. Crude castor oil was supplied by Cai Lay, Tien Giang, Vietnam, with a saponification index of 186.0 mg KOH/1 gram oil.

2.2. Extraction of Saponin from Soapnuts

The extraction of saponin from soapnuts was described elsewhere [6]. Briefly, the soapnut pericarp was cut and dried at 80 °C for three days in an oven, finely ground to powder, and then sieved with a stainless steel sieve (30 mesh) to remove larger particles. The extraction was performed using EtOH at the solid-solvent ratio of 10 g/100 mL. The mixture was stirred using a magnetic stirrer at 500 rpm for 6 hours at room temperature. The mixture was then vacuum filtered to get the extract containing saponins. The extract was stored in a refrigerator at 4 °C until used for analyses or further experiments.

2.3. Quantification of Total Saponin

The extraction saponin was qualified by a spectrophotometric method (the standard method for qualifying plant saponin [10]). This method was based on the reaction of oxidized saponins with vanillin by concentrated sulfuric acid to form a distinctive color of purple. First, the solution was prepared by adding saponin (11.0 g.L⁻¹), vanillin (wt. 8%), and H₂SO₄ (wt. 72%). After that, it was cooled with ice-cold water. Then, its absorption was measured with UV-Vis spectroscopy to determine the maximum absorption wavelength [11].

Saponin is extracted from natural products, so its concentration in the product is often variable in range, even for standard chemicals purchased from wellknown suppliers such as Sigma-Aldrich (Merck). Therefore, the determination of the composition of saponin in the extract is complex, and the result is often a mean value.

2.4. Preparation of Soap Base

The crude castor oil was filtered to remove any solid particulates. First, about 100 grams of the Castor oil was heated to 90 °C. Then an excessive amount (20 wt.%) of KOH in a 50 mL aqueous solution was gradually added to the oil. The reaction was carried out for 2 hours at 90 °C with an agitation of 200 rpm. Next, the mixture was aged at 130 °C for 24 hours. Then, 200 mL of hot water ($70 \div 80$ °C) was added to the product, followed by the addition of saponin extract and/or α -bisabolol essential oil. Finally, the product was agitated for 1 hour at 200 rpm until it appeared uniform. This final product is called soap base, then other ingredients (saponin and/or α -bisabolol) were added for further study.

2.5. Soap Analysis

Each of the prepared soaps was analyzed to determine several important detergency characteristics. A comparative analysis was also carried out using commercial soaps. The pH of all samples was measured at the storing state and after two months at room temperature. Other properties were determined after two months for stability.

pH of 1% sample solutions: The pH of 1 wt.% soap solution was measured at 25 °C using a HACH - sensION+ PH3 Lab pH-meter (US) and following Vietnamese standard No TCVN 5458:1991.

Foam height and stability: The foam height and foam retention were determined according to Vietnamese standard No TCVN 5817:1994.

The density of the samples: The densities of all samples were recorded following Vietnamese standard No TCVN 3731:2007.

Surface tension and critical micelle concentration (CMC):

Sample code	Compositions				
	Soap base (wt. %)	Saponin (wt. %)	A-bisabolol (wt. %) ⁽³⁾	Others (wt. %)	
Soap 1 ⁽¹⁾	-	-	-	100	
SP-Soap 1 ⁽²⁾	98	-	-	2 % CHG (4)	
SP-Soap 2 ⁽²⁾	100	0	0		
SP-Soap 3 ⁽²⁾	88.0	10	2.0		
SP-Soap 4 ⁽²⁾	88.5	10	1.5		
SP-Soap 5 ⁽²⁾	89.0	10	1.0		
SP-Soap 6 ⁽²⁾	89.5	10	0.5		

Table 1. Soap samples for testing microbial activity

⁽¹⁾ A commercial handwashing sample (Lifebuoy handwash)

⁽²⁾ Soap samples prepared from castor oils

⁽³⁾ The stock concentration of α -bisabolol: 45 wt.%,

⁽⁴⁾ CHG: antiseptic agent chlorhexidine gluconate

A Du Noüy ring tensiometer, model DST-60 (SEO company - Korea), measured the equilibrium surface tension of different concentration solutions to determine their CMC. The ring circuit was 6.1858 cm, and the ratio of the ring radius and its thickness (R/r) was 54.2857. Before each measurement, the ring was rinsed with acetone, then deionized water to remove any organic contaminants. The experimental temperature was 25 °C.

2.6. Stability of Soap Samples in Hard Water and Saline Water

The preparation of hard water model samples followed previous work [12]. Briefly, 0.441 g CaCl₂.2H₂O and 0.493 g MgSO₄.7H₂O were dissolved in 100 mL of deionized water to generate a stock solution equivalent to 5000 ppm CaCO₃. The preparation of water with different hardness (0 - 1000 ppm) was carried out by diluting an appropriate amount of the stock solution in deionized water. The soap samples were added to 10 mL of hard water so that the concentration was 1 gram of soap per 100 mL of hard water. The mixtures were shaken vigorously. The pictures of resulted samples were taken to record the turbidity of soap solutions in hard water.

Saline water model samples were prepared by dissolving NaCl in deionized water to form a series of NaCl solutions with a concentration range from 0.1 M to 1.0 M.

2.7. Anti-Bacterial Activity Test

Table 1 lists the codes for the tested samples and their components for bactericidal testing. The antibacterial and anti-microbial activities of soap samples were tested at the lab of The Military Institute of Forensic Medicine. The following reference strains were tested following their corresponding standard: Total Aerobic microbial count (ACM THA 06 Test Method); Staphylococcus aureus test (ISO 22717: 2015); Pseudomonas aeruginosa test (ISO 22718: 2015) and Candida albicans (ISO 18416: 2015).

3. Results and Discussion

3.1. Determination of Saponin Concentration

Calibration curve building

Fig. 2 shows the UV-Vis spectroscopy scanning of the solution series which were prepared by adding the various quantity of saponins + vanillin (8 wt. %) + sulfuric acid (72 wt. %). The result shows three peaks with maximum absorption at 450, 544, and 600 nm of wavelength. The $\lambda_{max} = 450$ or 544 nm was also chosen as the specific wavelength for the solution containing saponin, vanillin, and H₂SO₄. Thus, the $\lambda_{max} = 544$ nm was selected as the reference wavelength for building the calibration curve. The solution compositions for determining the calibration curve were prepared as



Fig. 2. The UV-Vis spectroscopy of saponin in a vanillin + H₂SO₄ solution

shown in Table 2, and the saponins solution was prepared from standard chemicals (purchased from Sigma-Aldrich).

Table 2. The components composition of samples to determine the calibration curve.

No.	Saponin, g.L ⁻¹	Vanillin, wt. %	H ₂ SO ₄ , wt. %
1	22,5	8	72
2	15,0	8	72
3	11,25	8	72
4	7,5	8	72
5	3,75	8	72

Table 3. The absorption value (A) of diluted extract solution (with the dilution factors).

No.	Saponin, g.L ⁻¹	Dilution factor, times	A, au
1	22,5	25	0.231
2	15,0	20	0.195
3	11,25	15	0.178
4	7,5	10	0.161
5	3,75	5	0.150

For determining the linear relationship between saponin concentration and UV-Vis absorbance, the absorption data of a series of saponin concentrations in reagent solutions were studied in a specific saponin concentration range. Then, based on this standard curve, the saponin concentration in the extract can be quantified.

Since the saponin concentration in the extracted solution was high, this solution was required to dilute before taking the UV-Vis spectroscopy measurement. Thus, it is important to dilute this solution sample with a dilution factor (using DI water) to get a suitable concentration of saponins, and then the UV-Vis spectroscopy could be performed. Table 3 shows sample data and dilution factors corresponding to the measured absorbance values.

Fig. 3 shows the calibration curve of saponin was conducted from the data in Table 2. Thus, the relationship between absorbance and concentration of saponin is expressed as the equation: $A = 0.0044 \times C_{saponin} + 0.1302$ with $R^2 = 0.0042$, indicating the linear relationship between these two parameters.

Results of saponin determination in the extract

The process of extracting saponins in the soapnut was used by liquid-solid extraction method using

ethanol solvent. The saponin in the extract was obtained from the extraction process of the dried soapnut (moisture < 1 wt.%) with a weight of 10.83 grams with 100 mL ethanol (99.5% v/v). The solid saponin was separated and dried from the extraction solution was 4.82 grams. Then, this dried saponin was dissolved in 100 mL of DI water to determine the total quantity of saponin using the calibration curve (Fig. 3). This saponin in soapnut was 7,91 wt.%. This saponin in the extraction was the additive material for preparing the bio-soap.



Fig. 3. Calibration curve of saponin in reagent solutions (vanillin 8 wt.% + H₂SO₄ 72 wt.%)

3.2. Characterization of Physicochemical Properties of Soaps.

The three soap samples (Soap 1; SP-Soap 1; SP-Soap 2, Table 1) were tested for various physicochemical properties. The results of three representative samples are shown in Table 4.

The pH of the soap base and final products is important because it must meet human health requirements or environmental aspects. The prepared soap bases and soap formulations are often expected to generate an alkaline pH medium. However, the higher pH values may cause skin irritation. In contrast, the lower pH of soap indicates the lack of alkaline during the soap-making process, which would cause a low saponification efficiency [13].

The pH values of the soap base (SP-Soap 2) and other soap formulations are from 9.0 to 9.5 (Table 4). All values are acceptable for different formulations of skin cleansing products without any further requirement for significantly adjusting the pH of the soap base [13]. The pH of all soap samples was measured again after 1 - 2 months, indicating some small change assigned to completing the saponification reaction.

Samples/Parameters	Soap 1	SP-Soap 2	SP-Soap 3
Appearance (1 % solution)	Clear, no color	Fat oily -like smell, Clear, pale light brown	Fat oily -like smell, Clear, pale light brown
pH curing state (1 % solution)	7.0	9.42	9.36
pH after 2 months (1 % solution)	7.0	9.03	9.01
Density (g/mL)	1.0321	1.0496	1.0198

Table 4. Physico-chemical properties of soap samples

The addition of α -bisabolol and/or saponin extract did not affect the pH values of the soap solutions because they are non-ionic compounds. The Saponin family is a series of non-ionic surfactants with a glycoside structure attached by some sugar units at different positions (Fig. 1). Therefore, the presence of this substance does not impact the pH of the soap solutions.

Among the different physicochemical properties of a surfactant, particularly for cleaning applications, the critical micelle concentration (CMC) is an important parameter. The CMC of surfactants is the threshold value of concentration at which the surfactant molecules can self-assemble into micelles in solution. At a concentration above the CMC value, more micelles can be generated. This value can be determined via the relationship between solution surface tension and surfactant concentration.

Surface tension measurement of different concentrations (from 0.02 to 0.1 g.L⁻¹) was carried out, and the obtained results are presented in Fig. 4. The surface tension profiles of all soap samples show a general pattern of the usual decrease of surface tension upon soap concentration increase. Besides, it can be observed that at all concentrations, the surface tension of SP-Soap 3 is relatively lower than SP-Soap 2. Measurements of a saponin sample and LAS were also performed for comparison, showing similar behavior that was reported [14].

Table 5. Surface tension and detergent properties of soap samples

Parameters	Soap 1	SP-Soap 2	SP-Soap 3
Foam height (mL)	330	240	280
Foam stability (%)	98.7	96.6	96.2
CMC (mg/L)	60.22	61.58	45.83
Surface tension (γ) at CMC (mN.m ⁻¹)	38.73	40.02	34.08
Surface pressure (π_{CMC}) at CMC, mN.m ⁻¹)	32.96	31.49	37.61

The plotting of the surface tension of these samples against the logarithm of their concentration (Fig. 5) presents a visible break. The CMC value is the concentration at the breaking point. The obtained CMC values and the surface tension at CMC of soap samples are determined and shown in Table 5. Surface tension at CMC of SP-Soap 3, being 34.08 mN.m⁻¹, is the least, and that of SP-Soap 2 (40.02 mN.m⁻¹) is the highest.



Fig. 4. Tension metric profiles of saponin, LAS, SP-Soap 2, and SP-Soap 3 in aqueous medium at 25 °C.



Fig. 5. Determination of critical micelle concentration of SP-Soap 2 and SP-Soap 3 in aqueous medium at $25 \,^{\circ}$ C and the corresponding linear least square fits. Those of other samples are omitted for clarity.

The presence of soap samples decreased the surface tension of water from 71.69 mN.m⁻¹ to values around 40.02 mN·m⁻¹ (at CMC). The surface tension of the sample containing saponin (SP-Soap 3) was 34.08 mN·m⁻¹, indicating the effect of saponin in lowering not only the CMC value but also the surface tension at the air-solution interface. Micelles only are generated when the soap concentration is higher than the CMC, and the more concentration of soap, the more micelles are formed. Therefore, the lower CMC could provide better micellization properties of natural soaps. Consequently, adding saponin extract enhances the cleansing properties of the soaps due to emulsion formation in water, which will help dissolve the dirt during the washing action.

From the CMC value, we can estimate the surface pressure at CMC (π_{CMC}) of a surfactant using the following equation [15]:

$$\pi_{CMC} = \gamma_o - \gamma_{CMC} \tag{1}$$

in which γ_0 and γ_{CMC} are the surface tension of water and samples at the CMC value.

The obtained surface pressures for SP-Soap 2, SP-Soap 3, and saponin were 31.95 mN.m⁻¹, 39.02 mN.m⁻¹, and 34.51 mN.m⁻¹, respectively. Thus, the presence of saponin in the soap base decreased the surface tension at the air-liquid interface, increasing surface pressure at CMC.

Surfactants are amphiphilic compounds with a hydrophilic head and a hydrophobic tail. The interaction between the surfactant head and water molecules results in the adsorption of surfactant molecules at the air-liquid interface so that their tails align toward the air. In addition, the saponin molecules, a non-ionic surfactant, are less polar than soap molecules, leading to the interaction of water molecules with hydrophilic heads of saponin molecules being weaker than those of soap molecules. Consequently, this reduces the surface tension and increases surface pressure.

Foam height and stability

The foam height within 60 seconds is comparable to foam formation ability, whereas that value after 3 minutes indicates the stability of the foam. The obtained results (in Table 5) of foam formation and foam stability indicate the effect of saponin in the soap base: The foam height increased while the stability of the foam stayed almost the same. It demonstrates the excellent ability of saponin to make foam in the aqueous environment. The presence of saponin lowered the surface tension of the soap sample (SP-Soap 3) at the concentration above CMC, resulting in higher surface pressure affecting the generated foam [16].

3.3. Evaluation of Soap Stability in Hard Water and Saline Water

Another critical parameter that strongly interferes with soap behavior is water hardness, mainly caused by calcium and magnesium ions. Hence, the effect of water hardness on soap samples was evaluated via their changes in appearance (cloud point), CMC values, and surface tension with different water hardness (0 – 1000 ppm, from right to left).

Fig. 6A presents pictures of the soap base in the presence and absence of saponin extract. The sample with saponin (SP-Soap 3) became turbid at a water hardness of 200 ppm, whereas the sample without saponin (SP-Soap 2) indicated cloudy at 50 ppm.

The precipitate generation and the mechanism for delaying the onset of precipitation can be explained as follows. The main components of these soaps are potassium salts of long-chain fatty acids (R–COOK). So, the negatively charged part of soap molecules tends to interact with positively charged magnesium ions (Mg²⁺) and calcium ions (Ca²⁺), generating a white precipitate [17].

$$N(R - COOK) + M^{n+} \rightarrow (R-COO)_n M \downarrow + nK^+$$

When saponin (a non-ionic surfactant) is added, a mixture of micelles is formed, decreasing the concentration of soap molecules in solutions. There is less interaction (positive charge reaction) of the cations (such as Ca^{2+} , Mg^{2+}) with anionic surfactants than with non-ionic surfactants. Hence, the driving force for precipitation is destructed, increasing hardness tolerance.



Fig. 6. Photographs of (A) SP-soap 2 and (B) SP-Soap 3 in different water hardness

Similar experiments were carried out to evaluate the effect of saponin on soap activities in saline water (Fig. 6B). The addition of saponin extract also enhances the stability of soap in the salt medium. Without saponin, the sample SP-Soap 2 was destroyed totally at a sodium chloride concentration of 0.1 M. At concentrated salt solution, the ionic strength is high, resulting in the so-called "salt-out effect." The consequence of this phenomenon is the precipitation in soap solutions. Besides, the presence of ionic salt shrinks the electric double layer, letting more soap molecules into micelles. As a result, it increases the size of aggregates and also causes precipitation.

The results of saponin's impact on stabilizing soap solutions are shown in Table 6. With the support

of saponin, soap sample SP-Soap 3 was still transparent and homogeneous until a concentration of NaCl reached 0.4 M. The mechanism of the soap solubility enhancement induced by saponin is complicated. Several reports have stated that non-ionic surfactant molecules support the screening of electrostatic interaction of the anion groups in the presence of ionic salt. In addition, like other non-ionic surfactants, saponin molecules contain no dissociable groups, so they are less affected by changes in ionic strength.

Saponin increases the stability of soap solutions in both hard and saline water, enhancing the use of soaps in severe conditions.

Observation	Stability test in hard water (*)		Stability test in saline water (**)		
	SP-Soap 2	SP-Soap 3	SP-Soap 2	SP-Soap 3	
Clear	< 50	< 200	< 0.1 M	$\leq 0.2 \text{ M}$	
Opalescent	50	200	-	-	
White gelatinous precipitate	> 100	> 400	≥ 0.1 M	≥ 0.4 M	

 Table 6. Observation of soap samples' stability in hard water and saline water

^(*) The unit of water hardness is equivalent to ppm CaCO₃

(**) Concentration of the NaCl solutions is in molarity (M)

Name of specific tests/	Unit	Microbial testing concentration	Total Aerobic microbial	Staphylococcus aureus	Pseudomonas aeruginosa	Candida albicans	Final
Sample codes		(control samples)	count				
Soap 1	count (CFU/mL)	5.1×10^4	48	2	3	3	Pass
	%		99.91	100.0	99.99	99.99	
SP-Soap1	count (CFU/mL)	6.2×10^4	35	3	4	2	Pass
	%		99.94	100.00	99.99	100.00	1 455
SP-Soap 2	count (CFU/mL)	6.8x10 ⁴	1700	45	47	46	Pass
	%		97.50	99.93	99.93	99.93	
SP-Soap 3	count (CFU/mL)	6.1×10^4	70	10	9	9	Pass
	%		99.89	98.98	99.99	99.99	
SP-Soap 4	count (CFU/mL)	5.9x10 ⁴	75	14	15	13	Pass
	%		99.87	99.98	99.97	99.98	
SP-Soap 5	count (CFU/mL)	6.0x10 ⁴	130	45	42	44	Pass
	%		99.78	99.93	99.93	99.93	1 455
SP-Soap 6	count	5.7x10 ⁴	160	55	51	53	
	(CFU/mL)						Pass
	%		99.72	99.90	99.91	99.91	

Table 7. Bactericidal activity of different soap samples

3.4. Bactericidal Activities

The prepared soap samples were evaluated for their anti-bacterial efficacy against various grampositive and gram-negative bacteria. The number of colonies after the incubation period was counted and given in Table 7. Compared to samples without antiseptic ingredients, the soap samples containing α-bisabolol or chlorhexidine gluconate (a commonly used anti-bacterial agent) demonstrate excellent bactericidal potential. Nevertheless, CHG somehow negatively influences humans [18], so natural essential oil a-bisabolol can be an efficient alternative. The bacterial activity in the soap samples that had α -bisabolol added met the required standard (>99.9%) for anti-bacterial activity. When comparing soap with remarkably similar samples efficiency, α -bisabolol concentration was two times lower than that of CHG. The reasonable ratio of α -bisabolol to the soap can range from 1.0 - 2.0 wt.%, and the suitable concentration of α -bisabolol in final products will be 1 - 1.5 wt.% for economic reasons.

4. Conclusion

This study indicates that adding saponin and essential oil α -bisabolol improves soap properties, such as foam height and foam stability, and lowers the surface tension at all concentrations.

In addition, the presence of saponin delays the precipitation of soap molecules in hard water as well as in brackish water thanks to the screening of electrostatic interaction. By the presence of 10 wt.% of saponin, the working limit of castor-oil-based soap salt extends from 200 ppm to 400 ppm water hardness and from 0.1 M to 0.2 M of NaCl in brackish water. The synergism created by combining numerous anionic surfactants and the action of additional non-ionic surfactants can lower the rate of anionic surfactant precipitation, allowing for more formulation flexibility in various applications.

Compared to ordinary soap, bio soap with added A-bisabolol has much better anti-bacterial properties. The anti-bacterial power is comparable to soaps containing chemical anti-bacterial additives such as chlorhexidine gluconate.

Acknowledgments

This research is funded by Hanoi University of Science and Technology under grant number T2020-PC-218

References

[1] K. G. O. Bezerra, I. G. S. Silva, F. C. G. Almeida, R. D. Rufino, L. A. Sarubbo, Plant-derived biosurfactants: Extraction, characteristics and properties for application in cosmetics, Biocatalysis and Agricultural Biotechnology, vol. 34, p. 102036, 2021/07/01/2021, https://doi.org/10.1016/j.bcab.2021.102036.

- [2] R. Jahan, A. M. Bodratti, M. Tsianou, and P. Alexandridis, Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications, Advances in Colloid and Interface Science, vol. 275, p. 102061, 2020/01/01/ 2020, https://doi.org/10.1016/j.cis.2019.102061.
- [3] J. Steber and H. Berger, Biodegradability of anionic surfactants, in Biodegradability of Surfactants, D. R. Karsa and M. R. Porter Eds. Dordrecht: Springer Netherlands, 1995, pp. 134-182.
- [4] M. J. Scott and M. N. Jones, The biodegradation of surfactants in the environment, Biochimica et Biophysica Acta (BBA)-Biomembranes, vol. 1508, no. 1-2, pp. 235-251, 2000.
- [5] S. Bhatta, L. R. Joshi, D. Khakurel, and R. W. Bussmann, Sapindus mukorossi Gaertn. Sapindaceae, in Ethnobotany of the Himalayas, R. M. Kunwar, H. Sher, and R. W. Bussmann Eds. Cham: Springer International Publishing, 2020, pp. 1-9.
- [6] M. D. Kose, O. J. W. J. o. R. Bayraktar, and Review, Extraction of saponins from soapnut (Sapindus Mukorossi) and their anti-microbial properties, World Journal of Research and Review, vol. 2, no. 5, p. 262952, 2016.
- [7] J. Lee, H. Jun, E. Jung, J. Ha, and D. Park, Whitening effect of α-bisabolol in Asian women subjects, Int. J. Cosmetic Sci., vol. 32, no. 4, pp. 299-303, 2010/08/01 https://doi.org/10.1111/j.1468-2494.2010.00560.x.
- [8] C. A. C. de Medeiros *et al.*, Evaluating the Antifungal Activity of α-Bisabolol in Association with NaCl on Fusarium oxysporum in Maize Grains, (in Eng), Curr. Microbiol., vol. 78, no. 2, pp. 604-610, Feb 2021, https://doi.org/ 10.1007/s00284-020-02313-8.
- [9] L. B. Eddin *et al.*, Health Benefits, Pharmacological Effects, Molecular Mechanisms, and Therapeutic Potential of α-Bisabolol, (in eng), Nutrients, vol. 14, no. 7, p. 1370, 2022, https://doi.org/10.3390/nu14071370.
- [10] C. Y. Cheok, H. A. K. Salman, and R. Sulaiman, Extraction and quantification of saponins: A review, Food Research International, vol. 59, pp. 16-40, 2014/05/01 https://doi.org/10.1016/j.foodres.2014.01.057.
- [11] S. Hiai, H. Oura, and T. Nakajima, Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid, Planta Medica, vol. 29, no. 02, pp. 116-122, 1976.
- [12] R. Awang, S. Ahmad, R. Ghazali, Properties of sodium soap derived from palm-based dihydroxysteatic acid, Journal of Oil Palm Research, vol. 13, no. 2, pp. 33-38, 2001. [Online]. Available: http://jopr.mpob.gov.my/wp- content/uploads/2013/0 9/joprv12dec2001-roila1.pdf.
- [13] S. Wolfrum, J. Marcus, D. Touraud, W. Kunz, A renaissance of soaps? — How to make clear and stable solutions at neutral pH and room temperature, Advances in Colloid and Interface Science, vol. 236, pp. 28-42, 2016/10/01/ 2016, https://doi.org/10.1016/j.cis.2016.07.002.

- [14] S. E. Anachkov, S. Tcholakova, D. T. Dimitrova, N. D. Denkov, N. Subrahmaniam, and P. Bhunia, Adsorption of linear alkyl benzene sulfonates on oil-water interface: Effects of Na+, Mg2+ and Ca2+ ions, Colloids Surf. Physicochem. Eng. Aspects, vol. 466, pp. 18-27, 2015/02/05, https://doi.org/10.1016/j.colsurfa.2014.10.059.
- [15] M. Abdul Rub, Aggregation and interfacial phenomenon of amphiphilic drug under the influence of pharmaceutical excipients (green/biocompatible gemini surfactant), PLOS ONE, vol. 14, no. 2, p. e0211077, 2019, https://doi.org/10.1371/journal.pone.0211077.
- [16] N. Yekeen, A. A. Malik, A. K. Idris, N. I. Reepei, and K. Ganie, Foaming properties, wettability alteration and interfacial tension reduction by saponin extracted from soapnut (Sapindus Mukorossi) at room and

reservoir conditions, J Pet Sci Eng, vol. 195, p. 107591, Dec 2020,

https://doi.org/10.1016/j.petrol.2020.107591.

- [17] C. H. Rodriguez, L. H. Lowery, J. F. Scamehorn, and J. H. Harwell, Kinetics of precipitation of surfactants. I. Anionic surfactants with calcium and with cationic surfactants, Journal of Surfactants and Detergents, vol. 4, no. 1, pp. 1-14, 2001/01/01 2001, https://doi.org/10.1007/s11743-001-0155-7.
- [18] A. Salimi, B. Alami, and J. Pourahmad, Analysis of cytotoxic effects of chlorhexidine gluconate as antiseptic agent on human blood lymphocytes, (in eng), Journal of Biochemical and Molecular Toxicology, vol. 31, no. 8, Aug 2017, https://doi.org/10.1002/jbt.21918.