บทความวิจัย

การพัฒนาเซรั่มผสมสารสกัดจากเปลือกผลไม้ที่มีฤทธิ์ยับยั้งเชื้อแบคทีเรียก่อโรคผิวหนัง

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บทคัดย่อ

ปัจจุบันผลิตภัณฑ์เซรั่มมีคุณสมบัติในการเพิ่มความชุ่มชื้นแก่ผิวหนัง อย่างไรก็ตามการพิจารณาประสิทธิภาพในการยับยั้งการเจริญเติบโตของจุลินทรีย์ที่ก่อให้เกิดโรคผิวหนังเป็นปัจจัยสำคัญที่ช่วยเพิ่มคุณค่าทางการใช้งานของผลิตภัณฑ์ดังกล่าว งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาเซรั่มที่มีฤทธิ์ต้านแบคทีเรียก่อโรคผิวหนัง โดยใช้สารสกัดจากเปลือกผลไม้ไทย 5 ชนิด ได้แก่ ทับทิม ทุเรียน ส้มโอ มังคุด และกล้วย ในการศึกษาความสามารถในการยับยั้งแบคทีเรียสายพันธุ์ *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* และ *Staphylococcus epidermidis* โดยทำการสกัดสารออกฤทธิ์ 2 วิธี ได้แก่ การสกัดด้วยน้ำและการสกัดด้วยเอทานอล 95% ผลการทดลองพบว่าเซรั่มที่มีส่วนผสมของสารสกัดเปลือกทับทิม ร้อยละ 1 สามารถยับยั้งการเจริญเติบโตของแบคทีเรียได้อย่างมีประสิทธิภาพมากที่สุด การประเมินความชอบพบว่าเซรั่มที่ผสมสารสกัดจากเปลือกทับทิมได้รับความนิยมสูงสุดในกลุ่มผู้เข้าร่วมการทดสอบ ดังนั้นสารสกัดจากเปลือกทับทิมจึงมีศักยภาพในการนำไปพัฒนาเป็นสารออกฤทธิ์หลักในผลิตภัณฑ์เซรั่ม เพื่อสุขอนามัยผิวหนังและอาจเป็นทางเลือกที่มีความปลอดภัยและยั่งยืนสำหรับการควบคุมการติดเชื้อจากจุลินทรีย์บนผิวหนังในอนาคต

คำสำคัญ: เปลือกผลไม้ การสกัด แบคทีเรียก่อโรคผิวหนัง เซรั่ม

การอ้างอิงบทความ: สุมนต์ทิพย์ คงตันจันทร์ฟัก, "การพัฒนาเซรั่มผสมสารสกัดจากเปลือกผลไม้ที่มีฤทธิ์ยับยั้งเชื้อแบคทีเรียก่อโรคผิวหนัง," *วารสารวิชาการพระจอมเกล้าพระนครเหนือ*, ปีที่ 36, ฉบับที่ 1, หน้า 1–14, เลขที่บทความ 261-088000, ม.ค.–มี.ค. 2569, doi: 10.14416/j.kmutnb.2025.11.001.

Research Article

Serum Development of Fruit Peel Extracts Against Skin Pathogenic Bacteria

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DOI: 10.14416/j.kmutnb.2025.11.001
Received 9 May 2025; Revised 3 August 2025; Accepted 5 September 2025; Published online: 5 November 2025

Abstract

Currently, serum products are widely recognized for their moisturizing properties. However, consideration of their antimicrobial efficacy against skin pathogens is also important to enhance the functional value of these products. This study aimed to develop serum products with antibacterial activity against common skin pathogens. The extracts in this study were obtained from the peels of five Thai fruits: pomegranate, durian, pomelo, mangosteen, and banana. The bacterial strains tested included *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The antibacterial activity of each extract and serum was evaluated. Two extraction methods were used: aqueous extraction and 95% ethanol extraction. The results showed that the serum containing 1% pomegranate peel extract exhibited the strongest antibacterial activity. Furthermore, the preference test revealed that most participants favored the serum containing pomegranate peel extract. Therefore, pomegranate peel extract may serve as a potential antibacterial ingredient in skincare serum products, contributing to improved skin hygiene and providing a safe and sustainable option for controlling microbial infections on the skin in the future.

Keywords: Fruit Peels, Extraction, Skin Pathogenic Bacteria, Serum

Please cite this article as: S. Kongtunjanphuk, "Serum development of fruit peel extracts against skin pathogenic bacteria," *The Journal of KMUTNB*, vol. 36, no. 1, pp. 1–14, ID. 261-088000, Jan.–Mar. 2026 (in Thai), doi: 10.14416/j.kmutnb.2025.11.001.

1. Introduction

Bacterial skin infections are primarily caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* can be either primary (affecting healthy skin) or secondary (occurring on damaged skin) [1], [2]. Common infections include cellulitis, erysipelas, impetigo, and folliculitis [3]. Other pathogens, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhimurium* also contribute to skin issues [4]–[6]. Factors like environmental conditions, skin immunity, and hydration play crucial roles in these infections, showing the importance of skin protection and nourishment.

Moisturizing serums have become popular for improving skin hydration, with ingredients like argan oil and hyaluronic acid significantly enhancing moisture levels [7], [8]. When paired with moisturizers, these serums effectively reduce dryness and improve skin texture [9]. Innovative formulations with probiotics, such as *Lactobacillus fermentum* CECT 5716, further enhance skin health [10].

The incorporation of antibacterial properties in skincare products is crucial for maintaining skin health and mitigating infections caused by skin-related bacteria. Probiotics and postbiotics have applied as alternatives to traditional antibiotics, promoting beneficial bacteria growth and inhibiting pathogenic bacteria. These agents can stimulate the immune system, enhance skin barrier components, and modulate inflammation [11]. Natural products like essential oils and honey have shown promise as antibacterial agents in wound dressings [12]. The skin microbiota plays a major role in maintaining homeostasis, and imbalances can lead to conditions such as eczema, psoriasis, and acne [13]. While topical antibiotics and antiseptics are commonly used to treat skin infections, increasing bacterial resistance and potential hypersensitivity reactions pose challenges [14]. Therefore, understanding the efficacy and resistance mechanisms of these agents is essential for their optimal use in skincare products.

Additionally, there is a growing interest in incorporating herbal products, especially from Thai fruits, into daily routines. Fruit peels and seeds can be transformed into herbal remedies, offering potential for development into dietary supplements, medicines, and cosmetics [15]–[21]. Utilizing these by-products reduces waste and promotes domestic herbal product development.

Extraction procedure of compounds from fruit peels commonly uses solvents such as water, ethanol, or mixtures of water and ethanol in varying proportions, depending on the desired compounds [22], [23]. This is due to the differing polarity requirements of each extractable constituent, with some compounds being water-soluble while others are soluble in ethanol. Additionally, certain fruit peels are coated with pectin on their surfaces, necessitating preliminary removal of pectin using organic solvents before subsequent extraction with water or ethanol [24].

This study aims to demonstrate the efficiency of the crude extract from five fruit peels in Thailand to inhibit skin pathogenic-related bacterial activity. These peels include pomegranate, durian, pomelo, mangosteen, and banana. The peels were extracted using 95% ethanol and deionized water as solvents. The obtained extracts were then assessed for their antibacterial activity against five skin pathogenic bacteria that cause skin infections: *Staphylococcus*

aureus, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Extracts demonstrating significant antibacterial activity will be further developed into herbal serum formulations for anti-skin-related bacterial infections.

2. Materials and Methods

2.1 Fruit Peel Preparation

Fruit peels used in this study include 1) pomegranate (*Punica granatum* L.), 2) durian (*Durio zibethinus*), 3) pomelo (*Citrus maxima* Burm.f. Merr.), 4) mangosteen (*Garcinia mangostana* Linn.), and 5) banana (*Musa sapientum* L.). The peels were cleaned, sliced, and dried at 60°C for 8 hours using a hot-air oven. After drying, they were placed in a desiccator and weighed until the weight was constant. Moisture content was determined following AOAC 2005 standards [25]. The dried materials were ground and sieved through a 30 mesh screen before extraction.

2.2 Fruit Peel Extraction

This study examined the extraction of herbal peels using aqueous and ethanol-based methods with shaking. For aqueous extraction, the maceration technique (conventional method) is applied. The 500 g of peels were combined with 2,500 ml of deionized water, shaken at 30°C, 50°C, and 80°C for 2, 4, 6, and 8 hours in an oil bath shaker incubator (MEMMERT WNB22), and then filtered and concentrated at 95°C to assess yield.

For the ethanol extraction, using maceration technique, 500 g of peels were mixed with 2,500 ml of 95% ethanol and shaken under the same temperature and time conditions. The liquid extract was filtered, and the solvent was removed with a rotary evaporator (BUCHI) before concentrating at 95°C and storing in 250 ml reagent bottles for further analysis, including Thin Layer Chromatography.

The crude aqueous extract was diluted with deionized water to achieve a concentration of 10 mg/ml. For the ethanol extract, it was diluted using dimethyl sulfoxide (DMSO) to a final concentration of 10 mg/ml. These extracts were stored in the microcentrifuge tube for subsequent experiments.

2.3 Thin Layer Chromatography (TLC)

Crude fruit peel extract samples were prepared in methanol at 1 mg/ml. Two microliters of each sample and standards gallic acid and rutin were spotted on a silica gel 60 GF254 plate. The stationary phase was developed using two mobile phase systems: S_1 (dichloromethane: methanol, 9:1) and S_2 (dichloromethane: methanol: water: acetic acid, 15:7:1:0.1), allowing solvent migration for 80 mm. After air-drying, the plate was visualized under visible light and UV-Vis at 254 and 366 nm, and R_f values were calculated using Equation (1).

$$R_f = \frac{\text{distance of the sample}}{\text{distance of the solvent}} \quad (1)$$

2.4 Bacteria and Cultural Media Preparation

The bacterial strains used in this study, *Escherichia coli* TISTR 780 (B_1), *Pseudomonas aeruginosa* TISTR 1467 (B_2), *Salmonella typhimurium* TISTR 1470 (B_3), *Staphylococcus aureus* TISTR 118 (B_4), and *Staphylococcus epidermidis* TISTR 1845 (B_5), were obtained from the Thailand Institute of Scientific and Technological Research (TISTR).

Nutrient Broth (NB) is a liquid medium that dissolves 13 g of NB powder (HIMEDIA®) in 1,000 ml of deionized water (DI) and sterilizes it at 121°C and 15 psi for 15 minutes. Plate Count Agar (PCA), used for bacterial colony counting, is prepared by dissolving 23.5 g of PCA powder (HIMEDIA®) in 1,000 ml of DI, sterilizing it, and pouring into Petri dishes to solidify under UV light. Mueller-Hinton Agar (MHA), for determining the Minimum Inhibitory Concentration (MIC), is made by dissolving 38 g of MHA powder (HIMEDIA®) in 1,000 ml of DI, sterilizing it, and allowing it to solidify in Petri dishes under UV light.

2.5 Bacteria Preparation

The experiment starts by culturing five bacterial strains in 200 ml of NB in 250 ml Erlenmeyer flasks, incubated at 37°C with shaking at 250 rpm for 18 hours.

Afterward, 1 ml of the culture is transferred to a new flask with 100 ml of fresh NB and incubated at the same conditions, measuring optical density at 600 nm hourly to determine maximum growth rate.

The culture is diluted with sterile deionized water for cell suspension preparation and spread onto PCA using serial dilutions from 10^{-1} to 10^{-10}. After incubating the plates at 37°C for 24 hours, colonies between 30 and 300 are counted to determine viable cell counts.

2.6 Antibacterial Activity

The antibacterial activity test evaluates fruit peel extracts and serum using the Agar Diffusion Method on MHA. A filtered crude extract is placed on a 6 mm diameter filter disc on an MHA plate inoculated with bacteria, incubated at 37°C for 24 hours, and the zone of inhibition is measured to assess antibacterial activity effectiveness. Volumes of 0, 10, 20, and 30 µl per disc are tested to identify the optimal quantity. After determining the minimum effective concentration, the MIC will be established by serially 10 fold diluting the extract from 10^{-1} to 10^{-15}, aiding potential applications.

2.7 Herbal Moisturizing Serum Development

The moisturizing serum formula utilizes antibacterial fruit peel extracts in both aqueous and ethanol forms. The most effective extract will comprise 1% by weight of the serum, alongside additional skin-nourishing ingredients.

The preparation involves dissolving disodium EDTA (0.10%w/w) and methyl gluceth-20 (3.50%w/w) in deionized water (78.28%w/w). Carbomer (0.15%w/w) is gradually added while stirring and heating to 75°C. Once heated, Butyl hydroxytoluene (0.05%w/w), Isopropyl myristate (8.50%w/w), PEG-20 methyl glucose sesquistearate (5.50%w/w), and Glycol stearate (1.00%w/w) are mixed in at 70°C. After cooling, the fruit peel extracts (1.00%w/w) and urea (1.00%w/w) mixture are incorporated, followed by thorough mixing with aminomethyl propanol (0.12%w/w). Finally, preservatives (0.50%w/w) and fragrances (0.30%w/w) are added to complete the formulation.

2.8 Stability of the Herbal Moisturizing Serum

The viscosity of the moisturizing serum will be assessed during stability testing, which involves two main categories: temperature and light effects. The temperature cycle will start at 28°C monthly, then

move to 4°C and 40°C before returning to 28°C to evaluate serum stability. After four months, viscosity will be measured using a viscometer (BROOKFIELD RVDVI+), and pH meter (Consort) levels will be measured. Additionally, color changes will be analyzed using a spectrophotometer (HunterLab ColorFlex EZ) after four months of exposure to fluorescent light at 28°C. The calculation of these changes is performed using Equation (2). These tests ensure the product's stability and safety.

$$\Delta E_{76} = \sqrt{\left(L_f^* - L_i^*\right)^2 + (a_f^* - a_i^*)^2 + (b_f^* - b_i^*)^2} \quad (2)$$

Where ΔE_{76} is the degree of color changes, L^* is a lightness, a^* is a red-green axis, and b^* is a yellow-blue axis at an initial and final stage of the color measurement.

2.9 Preference Test

The different formulations of moisturizing serums were evaluated through consumer preference testing using a 9-point hedonic scale. This assessment focused on color, scent, texture, and overall preference. The test involved a sample group of 30 individuals, consisting of an equal number of males and females, aged 18 to 60. Statistical data analysis was conducted using ANOVA and Duncan's Multiple Range Test (DMRT) tests, with SPSS version 16 software employed.

3. Results and Discussion

3.1 Growth Curve

The experimental results at 37°C demonstrated that each bacterial strain exhibited its maximum growth rate, with *E. coli* (B_1) and *S. typhimurium* (B_3) reaching 16 hours, while *P. aeruginosa* (B_2), *S. aureus* (B_4), and *S. epidermidis* (B_5) reached 17 hours as shown in Figure 1. This data will be used for further analysis.

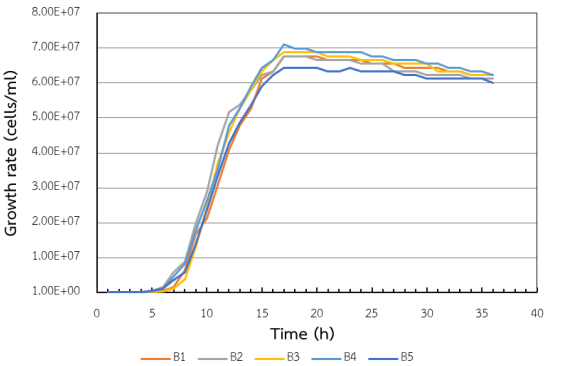


Figure 1 Bacterial growth rate.

3.2 Crude Extract

The extraction of various fruit peels revealed significant differences in the yield of extracts obtained using different solvents. Aqueous extraction produced significantly higher yields than ethanol extraction, with the average yield from aqueous extracts being approximately twice that of ethanol extracts after solvent evaporation (as shown in Table 1). This trend align with Chaiwarit *et al.* [22].

Factors influencing the yield and efficiency of extraction include the type of fruit, as different fruits possess varying concentrations of extractable compounds and solubility characteristics. Additionally, the freshness of the fruit peel plays a critical role, particularly in water and ethanol extraction methods. The use of fresh peel generally results in a higher percentage yield compared to dried peel.

Table 1 Yield of the crude extract

Crude Extract	Yield of the Extraction (%)	
	Aqueous (A)	Ethanol (E)
Pomegranate (1)	83.33	46.97
Durian (2)	33.33	11.80
Pomelo (3)	38.46	13.32
Mangosteen (4)	28.57	16.21
Banana (5)	37.04	3.73

3.3 Thin Layer Chromatography

Gallic acid (SM) is insoluble in water, making it undetectable in crude aqueous extracts but identifiable in ethanol extracts. In contrast, rutin, a flavonoid glycoside, is soluble in both water and ethanol, allowing its detection in both extracts.

S_2 exhibits increased polarity with a higher ratio of methanol to water than S_1, enhancing its ability to dissolve and transport bioactive compounds, resulting in higher Rf values. The Rf value of rutin in this study matches the 0.47 reported by Intarakasem *et al.* [26]. Similarly, the 0.11 Rf value for gallic acid found by Saxena *et al.* [27] aligns with this study. The greater polarity in S_2 facilitates better migration of gallic acid, leading to a higher R_f value than S_1.

Ethanol extracts of pomegranate (E1), durian (E2), pomelo (E3), and mangosteen peels (E4) showed Rf values matching gallic acid (SM), indicating its presence. Similarly, aqueous and all ethanol extracts of pomegranate (A1, E1), pomelo (A3, E3), mangosteen (A4, E4), and banana peels (A5, E5) showed Rf values matching rutin as standard marker (SM), as shown in Table 2, highlighting rutin as an essential bioactive compound in these extracts.

Table 2 Rate of flow (R_f) value of each extract

Extracts	R_f value			
	Gallic Acid		Rutin	
	S_1	S_2	S_1	S_2
SM	0.12	0.77	-	0.50
A1	-	-	-	0.50
A2	-	-	-	0.50
A3	-	-	-	0.50
A4	-	-	-	0.50
A5	-	-	-	0.50
E1	0.12	0.77	-	0.50
E2	0.12	0.77	-	0.50
E3	0.12	0.77	-	0.50
E4	0.12	0.77	-	0.50
E5	-	-	-	0.50

3.4 Antibacterial Properties

3.4.1 Crude Extracts

The experimental results indicated that only specific extracts demonstrated antibacterial properties. The active extracts included the aqueous extract from mangosteen peel (A4) and ethanol extracts from pomegranate (E1), durian (E2), pomelo (E3), and mangosteen peels (E4) at 30 µl. Notably, the ethanol extract from pomegranate peel (E1) exhibited the highest antibacterial efficacy. Detailed results are shown in Table 3, illustrating the inhibition zone sizes for the extracts.

In this study, only the aqueous mangosteen peel extract (A4) demonstrated antibacterial activity. According to Rizaldy *et al.* [28] has reported that mangosteen extract contains rutin, a compound known for its antioxidant properties. While the aqueous extracts from other fruit peels did not show antibacterial effects against skin pathogens, they similarly exhibited antioxidant activity comparable to

that of the aqueous extract of mangosteen peel (A4).

Table 3 Inhibitory zone of each extraction

Extracts	Inhibitory Zone (mm.)				
	B_1	B_2	B_3	B_4	B_5
A1	6.00	6.00	6.00	6.00	6.00
A2	6.00	6.00	6.00	6.00	6.00
A3	6.00	6.00	6.00	6.00	6.00
A4	14.33	14.33	13.66	16.33	15.00
A5	6.00	6.00	6.00	6.00	6.00
E1	22.00	22.66	21.66	22.66	33.00
E2	14.00	15.33	16.33	6.00	14.66
E3	14.66	17.66	18.00	18.66	11.33
E4	18.66	19.33	18.33	18.66	23.33
E5	6.00	6.00	6.00	6.00	6.00

Banana peel also contains tannins as one of its constituents. However, effective inhibition of bacterial growth by tannins requires a higher extract concentration than that used in this study. Consequently, the banana peel extract did not show antibacterial activity against *S. aureus* and *E. coli* [23].

In contrast, all ethanol extracts from fruit peels (E1-E4), except banana peel extract (E5), exhibited antibacterial activity. This can be attributed to the solubility of ethanol's key bioactive compounds, such as gallic acid and rutin. This solubility enhances their extraction and bioavailability. As a result, ethanol extracts demonstrated more substantial antibacterial effects than aqueous extracts due to the more efficient extraction of these bioactive compounds.

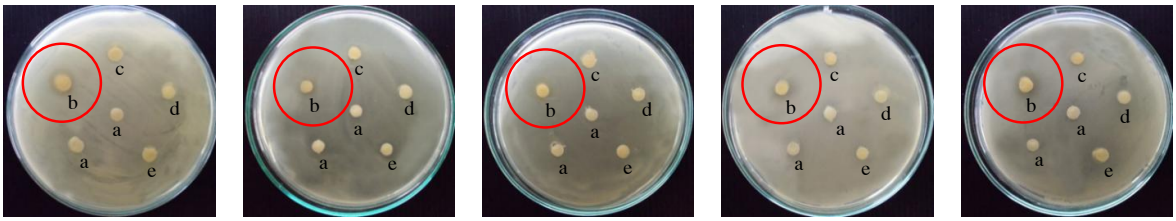
In the MIC experiments, the extracts tested included ethanol extracts from pomegranate (E1), durian (E2), and pomelo peels (E3), along with the aqueous extract of mangosteen peel (A4). The results showed that ethanol extract of pomegranate peel (E1) had the lowest concentration needed for antibacterial activity, outperforming the other extracts. These findings are summarized in Table 4, which compares the concentrations required for bacterial inhibition.

Table 4 Minimum inhibitory concentration (MIC) of each extraction

Extracts	MIC (mg/ml)				
	B_1	B_2	B_3	B_4	B_5
A1	-	-	-	-	-
A2	-	-	-	-	-
A3	-	-	-	-	-
A4	1×10^{-6}	1×10^{-6}	1×10^{-6}	1×10^{-5}	1×10^{-4}
A5	-	-	-	-	-
E1	1×10^{-10}	1×10^{-10}	1×10^{-10}	1×10^{-10}	1×10^{-13}
E2	1×10^{-5}	1×10^{-5}	1×10^{-5}	-	1×10^{-2}
E3	1×10^{-6}	1×10^{-5}	1×10^{-5}	1×10^{-2}	1×10^{-3}
E4	1×10^{-7}	1×10^{-8}	1×10^{-7}	1×10^{-7}	1×10^{-10}
E5	-	-	-	-	-

3.4.2 Moisturizing Serum

The study on the antibacterial properties in products containing 1% by weight of fruit peel extracts found that the inhibitory efficacy of most extracts decreased when incorporated into the product. Only the ethanol extract from the pomegranate peel (E1) retained its antibacterial properties. The aqueous extract from mangosteen peel (A4) was effective against only some bacterial strains, while the ethanol extracts from durian (E2) and pomelo peels (E3) cannot inhibit bacterial growth. The results of these tests are summarized in Table 5 and illustrated in Figure 2.



B_1 B_2 B_3 B_4 B_5

Figure 2 Inhibitory zone of serum: Control (a), E1 (b), E2 (c), E3 (d), and A4 (e).

Table 5 Inhibitory zone of each serum

Serum	Inhibitory Zone (mm.)				
	B_1	B_2	B_3	B_4	B_5
Control	6.00	6.00	6.00	6.00	6.00
E1	10.33	10.33	10.33	10.00	9.00
E2	6.00	6.00	6.00	6.00	6.00
E3	6.00	6.00	6.00	6.00	6.00
A4	6.00	6.00	10.33	10.33	8.00

The experimental results show that the serum formulation with pomegranate ethanol extract (E1) maintained antibacterial efficacy against all five bacterial strains tested. In contrast, extracts from other fruit peels lost their antibacterial activity, possibly due to the formulation's low 1% concentration.

These experimental results are consistent with the MIC test in Table 4, as the ethanol extract from pomegranate peel (E1) exhibited the lowest inhibitory concentration at 10-13 mg/ml, significantly different from other extracts. This could explain why the serum mixed with the ethanol extract from pomegranate peel (E1) retained its antibacterial efficacy even at low concentration.

3.5 Stability of the Herbal Moisturizing Serum

The product's stability test results showed that temperature affects its viscosity. Cyclic temperature changes led to an increase in viscosity, preventing separation and maintaining product's effectiveness. These results are presented in Table 6.

The product's color change was slight when stored at 28°C and exposed to fluorescence light. This is attributed to the fact that the product's main ingredients are derived from natural extracts. However, this color change was not significant. The results are shown in Figure 3 and Table 7.

Table 6 Stability of the serum

Serum	Stability			
	Initial viscosity (cP)	Final viscosity (cP)	Initial pH	Final pH
Control	48,250	75,250	5.17	5.00
E1	30,500	66,500	5.23	5.45
E2	31,000	74,500	5.67	5.55
E3	25,000	66,000	6.00	5.93
A4	66,000	74,750	6.08	6.17

The color change of each serum can be categorized into three groups: not perceptible ($\Delta E < 2$), slightly changed ($2 < \Delta E < 10$), and significantly changed ($10 < \Delta E$). According to the results, the Control serum and the A4 formulation are in the "not perceptible" group, which means each serum is the most stable against fluorescence light,

Figure 3 Color change of each serum.

Table 7 Serum color changes

Serum	Color measurement						ΔE_{76}	Changing
	L_i^*	a_i^*	b_i^*	L_f^*	a_f^*	b_f^*		
Control	95	–2	4	94	–1	3	1.73	Not perceptible
E1	80	–6	47	78	–3	30	17.38	Significant
E2	86	–3	18	86	–1	10	8.25	Slightly
E3	78	–2	17	79	–1	7	10.10	Significant
A4	86	–1	10	85	–1	10	1.00	Not perceptible

followed by the E2 formulation. It has been noted that E2 was classified in the "slightly changed" group, with its color fading. E1 and E3 are the least stable against fluorescence light, with their color fading from vivid to pale.

3.6 Preference Test

According to the ANOVA analysis, there is a significant difference both between and within the groups with *p*-value (Sig.) < 0.05, as shown in Table 8. Moreover, Duncan's Multiple Range Test (DMRT) revealed that the color factor indicated the serum containing the aqueous extract of mangosteen peel (A4) as the most satisfying formulation. Nevertheless, other factors (scent, texture, and overall preference), the most satisfying formulation is the serum containing ethanol extract from pomegranate peel (E1), as shown in Table 9. Therefore, formulation E1 is the most efficacious for antibacterial activity, and also a satisfying formulation.

Table 8 ANOVA analysis

Color	Sum of Squares	df	Mean Square	F	Sig.
Between groups	1137.058	4	284.264	790.569	0.000
Within groups	347.704	967	0.360		
Total	1484.761	971			
Scent					
Between groups	984.451	4	246.113	785.476	0.000
Within groups	249.723	797	0.313		
Total	1234.175	801			
Texture					
Between groups	1171.458	4	292.864	470.004	0.000
Within groups	571.393	917	0.623		
Total	1742.850	921			
Overall preference					
Between groups	977.526	4	244.382	504.552	0.000
Within groups	460.620	951	0.484		
Total	1438.146	955			

Table 9 Duncan's Multiple Range Test (DMRT) analysis

Color							Scent						
Serum	N	Subset for alpha = 0.05					Serum	N	Subset for alpha = 0.05				
		1	2	3	4	5			1	2	3	4	5
E2	30	5.39					E3	30	4.00				
Control			5.53				Control			4.80			
E3				6.31			A4				5.14		
E1					7.29		E2					5.86	
A4						8.18	E1						7.20
Sig.		1.000	1.000	1.000	1.000	1.000	Sig.		1.000	1.000	1.000	1.000	1.000
Texture							Overall preference						
Serum	N	Subset for alpha = 0.05					Serum	N	Subset for alpha = 0.05				
		1	2	3	4	5			1	2	3	4	
Control	30	4.74					Control	30	5.53				
E2			5.68				E3			5.76			
A4				6.28			E2			5.85			
E3					6.48		A4				7.02		
E1						8.11	E1					8.10	
Sig.		1.000	1.000	1.000	1.000	1.000	Sig.		1.000	0.209	1.000	1.000	

However, E1 is the least stable (as determined by the colorimetric method) compared to the others. There is an alternative to protect its stability by using an amber bottle to minimize the fading effect of the extract when exposed to fluorescent light.

4. Conclusions

The ethanol extract from pomegranate peel (E1) showed the most potent antibacterial activity (10–13 mg/ml), confirmed by MIC results. Most extracts lost antibacterial efficacy when added to the moisturizing serum, but the pomegranate ethanol extract (E1) retained its effectiveness against all strains. These extracts can be further developed for their potential application, such as scalp serum, body lotion, and soap.

Switching storage temperatures increased viscosity without product separation and exposure to fluorescent light caused minimal color changes. Overall, participants were satisfied with the moisturizing serum containing pomegranate ethanol extract.

5. Acknowledgements

This research was funded by Faculty of Applied Science, King Mongkut's University of Technology North Bangkok (KMUTNB) research grant (contact No. 653099). Moreover, I would like to express my gratitude to Dr. Juthamas Sangsai, Nuttatida Promsri, Ratichon Tiandee, and Satharat Pianfuengfoo for their contributions to the success of this research.

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