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Anti-Sebum Efficacy of *Phyllanthus emblica* L. (Emblica) Toner on Facial Skin

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Abstract: Oily skin is a problem for a large number of people, especially in tropical countries. This condition results in comedones, inflammatory acne, and other aesthetic problems in the skin. Emblica (*Phyllanthus emblica* L.) has a number of benefits for the skin; for instance, there were some studies that suggested that emblica has skin whitening effects, and anti-collagenase and anti-elastase activities; however, its anti-sebum efficacy has not been reported.The objective of this research was to study the anti-sebum efficacy of emblica toner on facial skin. The toner base was formulated, accelerated stability was tested, and preferences were evaluated in 10 volunteers. The toner base with the highest preference score was mixed with emblica extract. Then, the toner base and emblica toners were assessed for skin irritation by a single patch test in 30 volunteers. The anti-sebum efficacy was conducted using the randomized, single-blind, placebo-controlled, split-face method with unwashed and only-washed skin positions added to the middle of the forehead in the same group of volunteers assessed by a skin sebum measurement, SebumScale®, at 1 h before the test, once after washing, and 1, 2, 3 and 4 h after applying the toners on forehead and cheek skin. The stable toner base with the highest preference (85.6 ± 1.8%) was mixed with 1%, 2%, and 3% emblica extract. The toners were stable and did not cause any skin irritation. The 3% emblica toner was chosen for efficacy evaluation. The casual sebum levels of the forehead skin and cheek skin were 66.66 ± 7.01 and 56.12 ± 7.75 μg/cm^2, respectively. The sebum level of the unwashed skin position changed (5.0 ± 1.66%) insignificantly up to 4 h ($p > 0.05$). In comparison, the sebum level of the only-washed skin position was recovered to the casual sebum level (99.4 ± 1.23%) within 3 h. Furthermore, the anti-sebum efficacy of the emblica toner (23.5 ± 1.24%) was higher than that of the toner base (12.0 ± 1.52%) ($p < 0.05$). The anti-sebum efficacy of emblica toner on cheek skin (26.9 ± 1.78%) was higher than that on forehead skin (20.1 ± 1.34%) ($p < 0.05$). In summary, the model of evaluation of anti-sebum efficacy used in this study has been found to be practical, and the emblica toner is safe and has apparent anti-sebum efficacy on facial skin.

Keywords: anti-sebum; *Phyllanthus emblica* L.; toner; skin

1. Introduction

Oily facial skin may cause skin issues in a large number of people [1]. Typically, the skin appears greasy and dirty, and sebum is overproduced that causes several dermatological complications, including acne and seborrheic dermatitis [2,3], and also significantly affects the quality of life [4,5]. There have been a vast variety of oily facial treatments including cosmeceutical products that are easy

to broadly use externally [6]. Therefore, controlling oil is an essential key to cosmeceutical product development for these circumstances.

In the past, skin toner was a typical product used as a second cleansing agent for removing residual makeup after regular facial cleansing or used for removing excess sebum secreted from facial skin to prepare the skin before nourishing treatment. Toners may be categorized into alcohol-based or non-alcohol-based toners for various skin types such as oily skin, sensitive skin, or combination skin [7]. Nowadays, the diversity and prevalence of the products cause skin toners to be utilized more as cosmeceutical products with several purposes; for example, rehydrating skin, balancing skin pH, tightening skin pores, relieving irritation, and also antisepsis. Significantly, because of the growing awareness and demand of natural products, plants have gradually been used much more in cosmeceutical products [8]. Hence, toners that are developed as cosmeceutical products by adding therapeutic benefits of herbal extracts from plant's leaves, twigs, bark, or fruits for controlling oiliness tend to be popular.

Emblica, *Phyllanthus emblica* L. is a tree of the family Phyllanthaceae that is native to Southeast Asia and can be found in China and India. It is also called Indian gooseberry. In Thailand, it is known as Ma Kham Pom. The tree is small to moderate, reaching 1–12 m in height, and has 10–20 cm long deciduous branchlets with light green feathery leaves, greenish-yellow flowers, and nearly spherical, light greenish-yellow fruits [9,10]. Emblica has been used for internal and external applications since ancient times [11,12]. In addition, emblica is well-known for its high antioxidant content compared to vitamin C [13], which results in a skin whitening effect, and other aesthetic benefits including anti-collagenase and anti-elastase [14]. Moreover, emblica contains several phenolic compounds, including a large amount of tannin [15,16], which has an astringent effect [17] and an inhibitory effect on 5α-reductase, abilities that can reduce the skin sebum secretion [18,19]. The phenolic hydroxyl groups in tannin have an essential role in the astringent effect by bringing about protein complexes [20–22]; as a result, plants containing tannin, including apple peel, green tea, and guava leaves, have been studied for their anti-sebum activity [23–25]. So far, there have been no studies about the clinical efficacy of emblica fruits in terms of anti-sebum activity.

Therefore, the present research aimed to develop and study the anti-sebum efficacy of toner containing emblica fruit extract (emblica toner) on facial skin in volunteers with oily/combination facial skin using clinical assessment and skin bioengineering measurements.

2. Materials and Methods

2.1. Plant Material Collection and Authentification

The emblica fruits were purchased from Mueang Mai Market in ChiangMai province, Thailand. The plant material was identified with the herbarium specimen and kept as a voucher specimen, number (ICHNC 101), in the herbarium at Faculty of Pharmacy, ChiangMai University, Thailand.

2.2. Emblica Fruit Extract

The fresh emblica fruits were dried at 60 °C for 3 days. For the aqueous extraction, 100 g of dried emblica fruits were soaked in a container of 500 cm^3 of deionized water (DI water) for 1 h, and then heated until the water boiled and simmered for 1 h. Afterwards, the emblica solution was filtered with gauze and cotton, while the remaining emblica fruits were left in the container. The emblica fruits and their residue were repeatedly extracted 2 more times. The first, second and third emblica solutions were mixed and filtered with Whatman® filter paper grade 4. The emblica filtrate was concentrated by evaporating with a rotary evaporator to remove the solvent and added with deionized water to obtain 100 g of emblica crude extract, which was then stored in a controlled refrigerator at 8 °C. The emblica extract was prepared by diluting 10 g of the emblica crude extract with deionized water to obtain 100 g of emblica extract. Ultimately, the emblica extract was stored at room temperature.

2.3. Total Tannin Content

Total tannin content of emblica fruit extract was measured using the Tannin Microplate Assay Kit (Catalog # CAK1060) that was acquired from Cohesion Biosciences (London, UK) and analyzed using the Microplate Reader (Model: SpectraMax M3) from Molecular Devices (San Jose, CA, USA). According to the manufacturer's instructions, it is recommended to warm the reaction buffer and the dye reagent to room temperature before adding deionized water and the sample or the standard reagent into the microplate. The substances were mixed and left at room temperature for 10 min. Then, the mixture was measured and its absorbance was recorded at 650 nm. Finally, the total tannin content of the sample was calculated from the standard curve.

2.4. Formulation and Stability Evaluation

The emblica toner was developed from toner base, which was composed of allantoin, glidant trithanolamine (Namsiang, Thailand), trimethylglycine (Sinthai, Thailand) and deionized water (RCI Lab Scan, Thailand) and formulated with different proportions of glycerin and water (Table 1). The pH value of toners was measured by pH meter (Model: ST3100, OHAUS, Parsippany, NJ, USA). Moreover, the 3 toner base formulas were tested for their stability by a centrifugation assay (Model: Sorvall Super T21 Benchtop Centrifuge, GMI, Ramsey, MN, USA) with 3000 rpm for 30 min at 25 °C, and 6 cycles of accelerated testing (heating and cooling at 4 °C and 45 °C for 48 h of each cycle) [24,25]. The most preferred formula, selected by conducting preference tests of the formulas, which passed the previous stability test, was mixed with different concentrations (1–3%) of emblica extract that were also tested for their stability again, as mentioned earlier. After that, the derived toner base and emblica toners were used for the skin irritation test. Eventually the non-irritating stable emblica toner with a high concentration of emblica extract was selected for anti-sebum efficacy evaluation.

Table 1. Development of the stable emblica toners.

Ingredient	Toner Base (%, *w*/*w*)			Emblica Toner (%, *w*/*w*)		
	A	**B**	**C**	**B1**	**B2**	**B3**
Glycerin (humectant)	1	2	4	2	2	2
Alantoin (anti-irritant), Trimethylglycine (anti-inflammatory), and Glidant (preservative)	3.1	3.1	3.1	3.1	3.1	3.1
DI water (solvent)	95.9	94.9	92.9	93.9	92.9	91.9
Triethanolamine (pH adjuster)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Emblica extract (active substance)	-	-	-	1	2	3
pH						
Intial	6.02 ± 0.03	6.00 ± 0.02	6.01 ± 0.02	5.68 ± 0.02	5.63 ± 0.02	5.59 ± 0.03
Heating and Cooling	5.95 ± 0.03	5.93 ± 0.02	5.87 ± 0.03	5.63 ± 0.02	5.56 ± 0.03	5.51 ± 0.03
Preference (%)						
Spreadability	80 ± 3.0 ^{a}	86 ± 3.1 ^{a}	80 ± 3.0 ^{a}			
Absorption	86 ± 3.1 ^{b}	90 ± 4.5 ^{b}	70 ± 4.5 ^{c}			
Greasiness	84 ± 4.0 ^{d}	88 ± 3.3 ^{d}	72 ± 3.3 ^{e}	NA	NA	NA
Color	82 ± 3.6 ^{f}	86 ± 3.1 ^{f}	78 ± 3.6 ^{f}			
Odor	70 ± 3.3 ^{g}	78 ± 5.5 ^{g}	68 ± 3.3 ^{g}			
Overall preference (%)	80.4 ± 1.7 ^{h}	85.6 ± 1.8 ^{h}	73.6 ± 1.7 ^{i}			

Different letters in the same row indicate statistically significant differences, ($p < 0.05$); NA: not available.

2.5. Preference Test

The preference test of toner base was conducted in 10 Thai volunteers, including 5 males and 5 female volunteers, aged 20–45 years old, by using a questionnaire with hedonic system scoring from 1 to 5 (dislike to prefer the most). The score was calculated as a percentage [26]. The stable toner base with the highest preference score was then used for the development of emblica toner.

2.6. Clinical Evaluation

2.6.1. Inclusion Criteria

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Suan Sunandha Rajabhat University (Approval code: 61-158-1-2), and written informed consent was obtained from all volunteers before they participated in the study.

Healthy Thai volunteers, with an oily/combination skin type following the study of Sang-Woong Youn [27], aged 20–45 years, were enrolled in this study. The inclusion criteria for the volunteers included an average facial casual sebum level of 40–100 μg/cm^2 (over 50 μg/cm^2 on the forehead and over 40 μg/cm^2 on the cheeks) measured by SebumScale®. All volunteers had no allergic skin conditions, no open wounds, and no skin inflammation. In addition, no volunteers were pregnant or breastfeeding. Significantly, all volunteers were not allowed to apply any oil control skincare products for 4 weeks before the beginning of the experiment, and not allowed to apply any products after the night before the experiment started.

2.6.2. Skin Irritation Test

The skin irritation test was conducted with the 30 volunteers who met the inclusion criteria. The 3 formulas of stable emblica toners and the toner base were comparatively examined in parallel with distilled (DI) water (negative control) and 3% sodium lauryl sulfate (positive control) as follows.

(1) Observing the irritation in skin and using a 5-point grading scale of increasing irritation (0 = no reaction, 0.5 = very weak erythema, 1 = weak erythema, 2 = marked erythema, and 3 = pronounced erythema [28]) and calculated Mean Irritation Index (MII).
(2) Measuring the erythema index of the irritated skin by using SkinColorCatch®, an instrument that detects the light reflecting from the skin with its RGB sensor to obtain the erythema index, acquired from Delfin Technologies (Kuopio, Finland), immediately before occlusion and after removal of the 24-h occlusion patches at 1, 24, 48, and 72 h [29].

2.6.3. Research Methodology: Split-Face, Randomized, Single-Blind Placebo-Controlled Study

The split-face, randomized, single-blind placebo-controlled study model [24,25,30] was adapted to study the anti-sebum efficacy of emblica toner. The volunteers were randomized to apply emblica toner on the left or right side of each of their faces and apply toner base on the other side with unwashed (nontreatment) and only-washed (control treatment) skin positions added to the middle of the forehead.

2.6.4. SebumScale®

The SebumScale® (Delfin Technologies Ltd., Kuopio, Finland) is a skin sebum measuring instrument based on quartz crystal microbalance. The quartz crystal sensor is placed on the skin to absorb the sebum, with a contact time of 5 s. The SebumScale® measures the mass of gathered sebum by analyzing changes in resonance frequency of the quartz crystal. SebumScale® is a non-invasive measurement device with a range of 0–150 μg/cm^2.This device has a resolution of 0.1 μg/cm^2 for 0–1 μg/cm^2; hence, it can measure tiny changes in sebum levels quite well. SebumScale® is a device that is appropriate to measure sebum level changes during treatment conditions.

2.6.5. Facial Sebum Measurement

SebumScale® can be used to assess skin sebum levels effectively. Moreover, SebumScale® has been used widely in a number of studies, including an analysis of skin sebum excretion performed with patients who received treatments with broadband light [31]. The measurements were made according to the manufacturer's instructions. The experiment was conducted in an air-conditioned, clean room to reduce the impact of confounding factors such as ambient temperature, humidity, daily rhythm,

and hormonal balance [32]. Usually, sebum recovery takes approximately 2–3 h after washing to the casual level [33]; therefore, the period of 4 h was set for this study to cover the period of sebum recovery.

2.6.6. Efficacy Evaluation

With the same group of volunteers, anti-sebum efficacy evaluation tests were conducted with the highest concentration emblica toner that was stable and non-irritating. After resting for 30 min to acclimatize to ambient conditions, the facial skin sebum levels of volunteers were measured at each position, designated as specified in Figure 1, at 23–25 °C and 40–60% relative humidity. Herein, the facial skin sebum level, which was measured 1 h before washing, was called the "Casual Level". The facial skin of each volunteer was cleaned with the same mild facial cleanser except the area covering the position F_0, and, after that (0 h), its sebum level was measured. The volunteers were randomized to apply 2 cm^3 of emblica toner on the left or right side of each of their faces by block randomization (from randomization.com) and to apply 2 cm^3 of toner base on the other side using cotton balls, except the area covering the position F_C, as specified in Figure 1. The sebum level of each position was measured again after 1, 2, 3, and 4 h, as shown in Figure 2, and then the sebum recovery and the anti-sebum efficacy was calculated as follows:

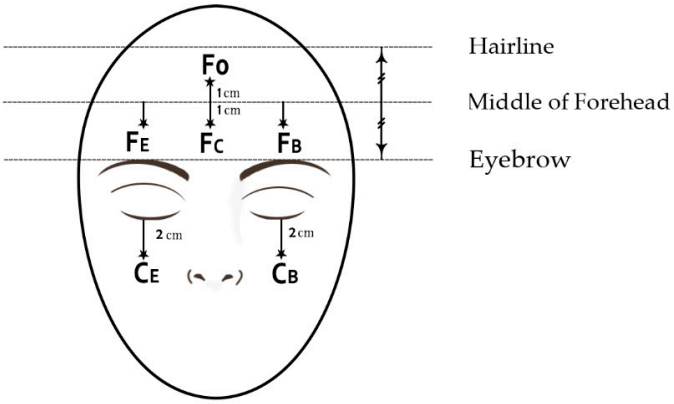


Figure 1. Positions on facial skin for specified methods of treatment. F_0 = unwashed (non-treatment) on forehead; F_C = washed only (control treatment) on forehead; F_B = washed + toner base on forehead; F_E = washed + emblica toner on forehead; C_B = washed + toner base on cheek; C_E = washed + emblica toner on cheek.

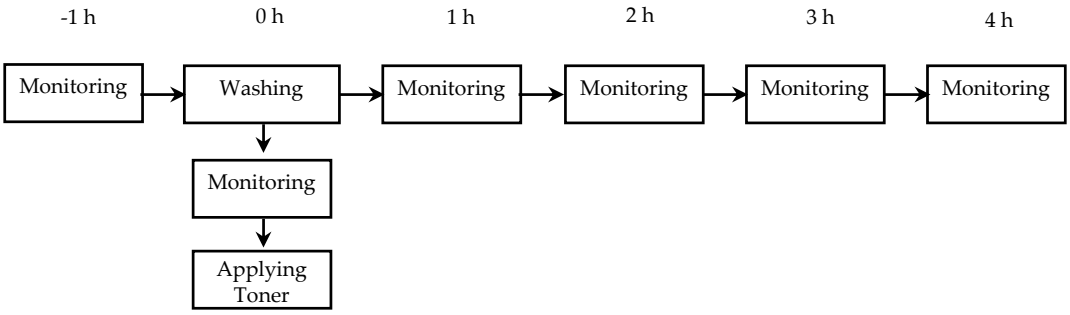


Figure 2. Steps in the experiment.

The sebum recovery of facial skin for treatment X at time t ($SR_{X,t}$) was calculated by comparing the facial sebum level with the casual level (baseline) of each position in percentage value:

$$SR_{X,\,t} = \frac{S_{X,\,t}}{S_{X,\,-1}} \times 100\%. \quad (1)$$

where:

$SR_{X,t}$ = Sebum Recovery after washing and applying Toner X at time t;
$S_{X,-1}$ = Sebum casual level at 1 h before treatment X was applied;
$S_{X,t}$ = Sebum level for treatment X at time t.

The anti-sebum efficacy of toner X at time t ($as_{X,t}$) was calculated by comparing the sebum recovery of toner X with the sebum recovery of washed only (control treatment) position at time t in percentage value:

$$as_{x,t} = \frac{SR_{C,t} - SR_{X,t}}{SR_{C,t}} \times 100\%. \quad (2)$$

where:

$as_{x,t}$ = Anti-Sebum Efficacy of toner X at time t (1, 2, 3, 4 h);
$SR_{C,t}$ = Sebum Recovery after being washed only (control treatment) at time t (1, 2, 3, 4 h);
$SR_{X,t}$ = Sebum Recovery after being washed and Toner X applied at time t (1, 2, 3, 4 h).

Since the sebum recovery changed over time, as shown in equation No. 2, anti-sebum efficacy of toner X on the facial skin over period t ($AS_{X,t}$) can be calculated by comparing the area under the curve (AUC) of the sebum recovery of toner X with that of the washed-only (control treatment) position of each period in percentage value:

$$AS_{X,t} = \frac{AUC(SR_{C,t})_{t-1}^{t} - AUC(SR_{X,t})_{t-1}^{t}}{AUC(SR_{C,t})_{t-1}^{t}} \times 100\% \quad (3)$$

where:

$AS_{X,t}$ = Anti-Sebum Efficacy of toner X over period t (1, 2, 3, 4 h);
$AUC(SR_{C,t})_{t-1}^{t}$. = AUC of Sebum Recovery after being washed only (control treatment) over period t − 1 to t (1, 2, 3, 4 h);
$AUC(SR_{X,t})_{t-1}^{t}$ = AUC of Sebum Recovery after being washed and Toner X applied over period t − 1 to t (1, 2, 3, 4 h).

2.7. Statistical Analysis

The preference test and the comparison of the means at different periods of each position (the irritation tests, the sebum level and the anti-sebum efficacy) were statistically analyzed using repeated measures of analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. The comparison of the sebum levels of different positions at the same time were statistically analyzed using one-way ANOVA followed by Bonferroni's post-hoc test. The anti-sebum efficacy evaluations were statistically analyzed using an independent *t*-test to compare 2 positions at the same time. The statistical significance was set to $p < 0.05$ and expressed as mean ± SEM. The statistical analysis was done with SPSS version 17.0.

3. Results

3.1. Emblica Extract

Total tannin content of the emblica extract was analyzed to be 40.56 ± 0.27 mg/g dried emblica.

3.2. Development of Emblica Toner

The development of emblica toners began with the preparation of toner base formulas A, B, and C containing 3.1% concentrations of a mixture of allantoin, trimethylglycine and glidant together with 1%, 2%, 4% concentrations of glycerin, respectively, with deionized water as a solvent. In addition,

trithanolamine was added to adjust the pH to be approximately 5.5–6.0, which suits the facial skin. After the stability test, the toner base formulas A, B, and C were found to be stable and homogeneous. The toner base with the highest preference score in 10 volunteers, including 5 males and 5 female volunteers, was chosen to be developed into the the emblica toner. It was found that the toner base formula B had the highest preference score, so it was mixed with varied emblica extracts of 1%, 2%, and 3%, respectively, as shown in Table 1.

3.3. Skin Irritation Test

The skin irritation test in 30 volunteers who met the inclusion criteria was conducted by a single closed patch test. By observing and calculating Mean Irritation Index (MII), it was found that the three formulas of emblica toners (B1, B2, B3) and the toner base (B) were negative, similar to deionized water (MII = 0 at 1, 24, 48, and 72 h), and contrary to the positive control, SLS (Sodium Lauryl Sulfate) (MII = 0.07, 0.18, 0.05, 0 at 1, 24, 48, and 72 h). By measuring with SkinColorCatch®, it was found that there were not statistically significant differences ($p > 0.05$) in the erythema indexes of different formulas of toners (B, B1, B2, B3) immediately before occlusion and after removal of occlusion at 1, 24, 48, and 72 h, as shown in Figure 3 and Table A1 (Appendix A); therefore, 3% emblica toner, the highest concentration toner used in the experiment, was selected for the anti-sebum efficacy evaluation test in the same group of 30 volunteers.

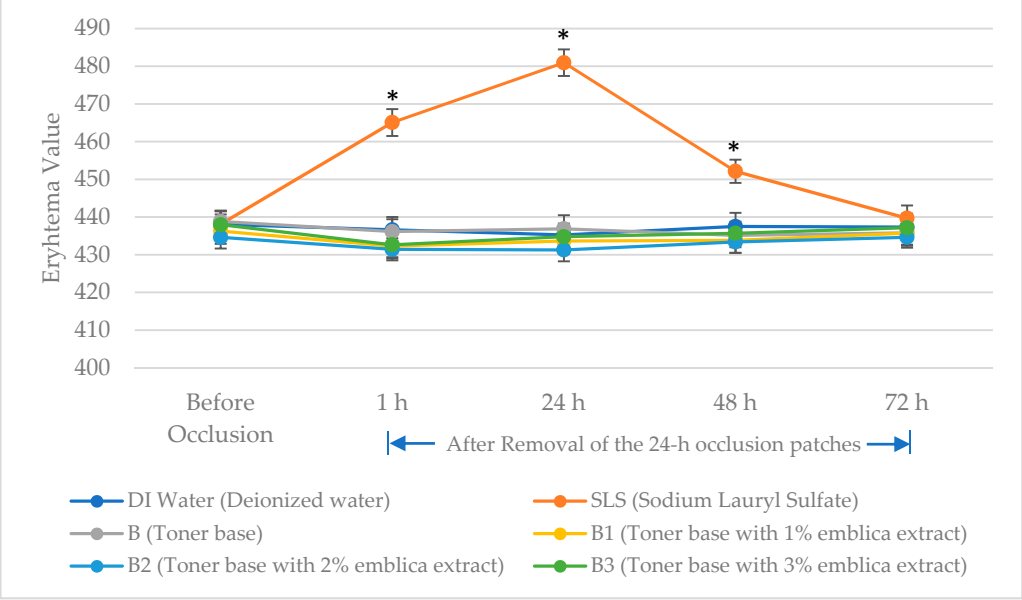


Figure 3. Erythema index of skin irritation test at different periods of 30 volunteers. * indicates statistically significant differences between erythema index after removal of the 24-h occlusion patches and before occlusion ($p < 0.05$).

3.4. Sebum Level

A total of 30 volunteers (16 male and 14 female volunteers; average age of 36 ± 6.29 years old, ranging from 20 to 45 years old) whose facial casual sebum level values were, on average, 63.14 ± 6.89 μg/cm^2, ranging from 50.5 to 79.5 μg/cm^2, with an average of 66.66 ± 7.01 μg/cm^2 and a range of 53.5–81.5 μg/cm^2 for forehead skin, and with an average of 56.12 ± 7.75 μg/cm^2 and a range of 42.5–75.5 for cheek skin, were included in the study.

The sebum level of the unwashed area (nontreatment) on forehead position F_0 at −1, 0, 1, 2, 3, and 4 h after the beginning changed statistically insignificantly ($p > 0.05$). It was found that the sebum levels in position F_C, F_B, F_E, C_B, C_E share the following common characteristics: the sebum level decreased after washing at 0 h, and then recovered gradually with a reducing rate from 0 to

3 h, and only F_C (washed-only position; control treatment) eventually became steady at the casual level (66.8 ± 1.37 μg/cm^2) from 3 to 4 h (66.5 ± 1.64 to 67.8 ± 1.73 μg/cm^2, respectively) ($p > 0.05$). However, the sebum levels in position F_B, C_B (64.1 ± 1.63, 53.5 ± 1.78 μg/cm^2, respectively) recovered to the casual level (66.4 ± 1.28, 55.9 ± 1.47 μg/cm^2, respectively) on each position at 4 h ($p > 0.05$), while the sebum levels in position F_E, C_E (58.2 ± 1.64, 48.1 ± 1.57, respectively) were still less than the casual level 66.5 ± 1.36, 56.3 ± 1.44 μg/cm^2, respectively) at each position at 4 h ($p < 0.05$), as shown in Table 2 and Figure 4.

Table 2. The sebum level on the forehead and cheek skin (F_C, F_B, F_E, C_B, C_E) at −1, 0, 1, 2, 3, and 4 h of 30 volunteers.

Time (h)	Sebum Level (μg/cm^2)					
	Unwashed (Nontreatment)	Washed Only (Control Treatment)	Washed			
			Toner Base		Emblica Toner	
	Forehead (F_0)	Forehead (F_C)	Forehead (F_B)	Cheek (C_B)	Forehead (F_E)	Cheek (C_E)
−1	66.8 ± 1.41 ^{A,a}	66.8 ± 1.37 ^{B,a}	66.4 ± 1.28 ^{F,a}	55.9 ± 1.47 ^{K,b}	66.5 ± 1.36 ^{P,a}	56.3 ± 1.44 ^{V,b}
0	67.3 ± 1.40 ^{A,c}	2.5 ± 0.21 ^{C,d}	2.5 ± 0.22 ^{G,d}	2.0 ± 0.19 ^{L,d}	2.4 ± 0.21 ^{Q,d}	1.9 ± 0.19 ^{W,d}
1	67.8 ± 1.46 ^{A,e}	34.1 ± 0.80 ^{D,f}	30.9 ± 0.98 ^{H,fg}	21.4 ± 1.19 ^{M,h}	26.9 ± 0.81 ^{R,g}	18.3 ± 1.05 ^{X,h}
2	68.1 ± 1.40 ^{A,i}	61.4 ± 2.07 ^{E,i}	53.4 ± 1.98 ^{I,j}	40.3 ± 1.92 ^{N,kl}	45.1 ± 1.82 ^{S,k}	33.9 ± 1.58 ^{Y,l}
3	68.8 ± 1.20 ^{A,m}	66.5 ± 1.64 ^{B,mn}	61.4 ± 1.74 ^{J,no}	50.4 ± 1.88 ^{O,pq}	54.6 ± 1.84 ^{T,oq}	44.4 ± 1.76 ^{Z,p}
4	69.9 ± 1.35 ^{A,rs}	67.8 ± 1.73 ^{B,rt}	64.1 ± 1.63 ^{F,stu}	53.5 ± 1.78 ^{K,vw}	58.2 ± 1.64 ^{U,uw}	48.1 ± 1.57 ^{Ω,v}

Different capital letters ($^{A–Z,Ω}$) in the same column indicate statistically significant differences ($p < 0.05$) when compared within the same column. Different lower case letters ($^{a–w}$) in the same row indicate statistically significant differences ($p < 0.05$) when compared within the same row.

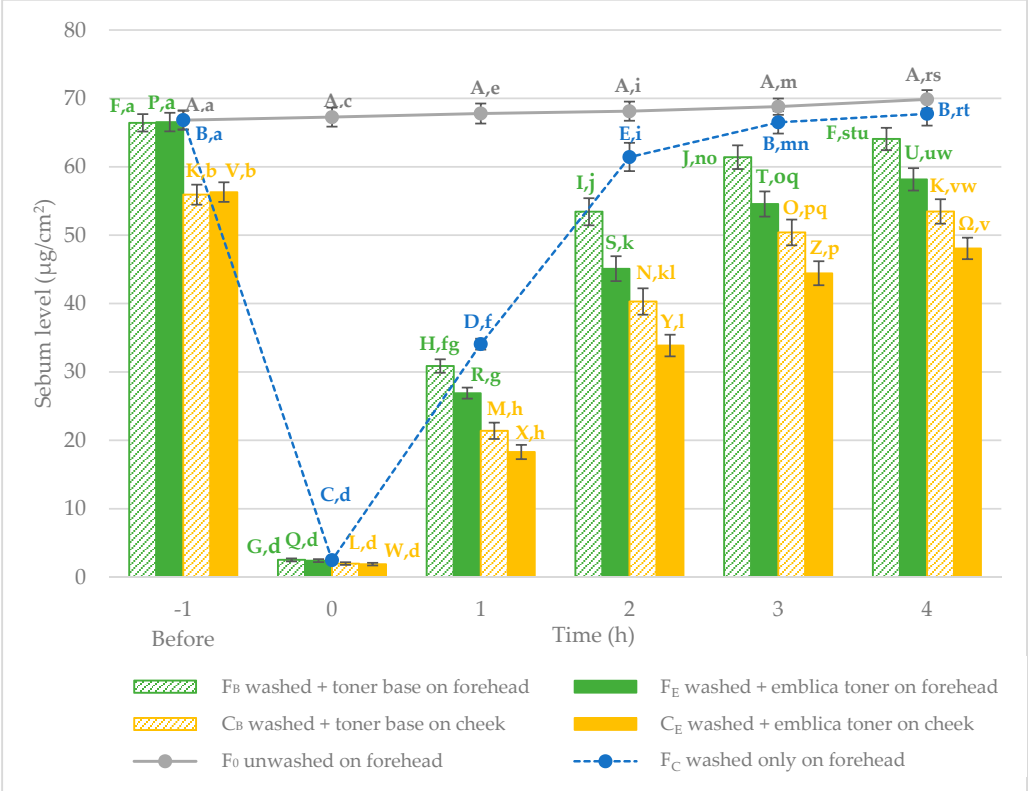


Figure 4. Sebum level on forehead and cheek skins of 30 volunteers at different periods. Different capital letters ($^{A–Z,Ω}$) indicate statistically significant differences ($p < 0.05$) in the same treatment among different periods. Different lower case letters ($^{a–w}$) indicate statistically significant differences ($p < 0.05$) in the same period among different treatments.

3.5. Sebum Recovery

The sebum recovery (SR) on the facial skin was calculated by Equation (1), as shown in Figure 5 and Table A2. Throughout the entire experiment, for unwashed position (F_0), there was a slight change to be 105.0 ± 1.66%. For position F_C, F_B, F_E, C_B and C_E, it was found that the sebum recovery decreased at 0 h and increased gradually from 0 h to 4 h, reaching 101.1 ± 0.95%, 96.2 ± 1.20%, 95.5 ± 1.73%, 87.2 ± 1.17%, 85.2 ± 1.37%, respectively. Consequently, the area under the curve (AUC) could be calculated using Equation (3) with the data from Table A2, as shown in Figure 6 and Table A3.

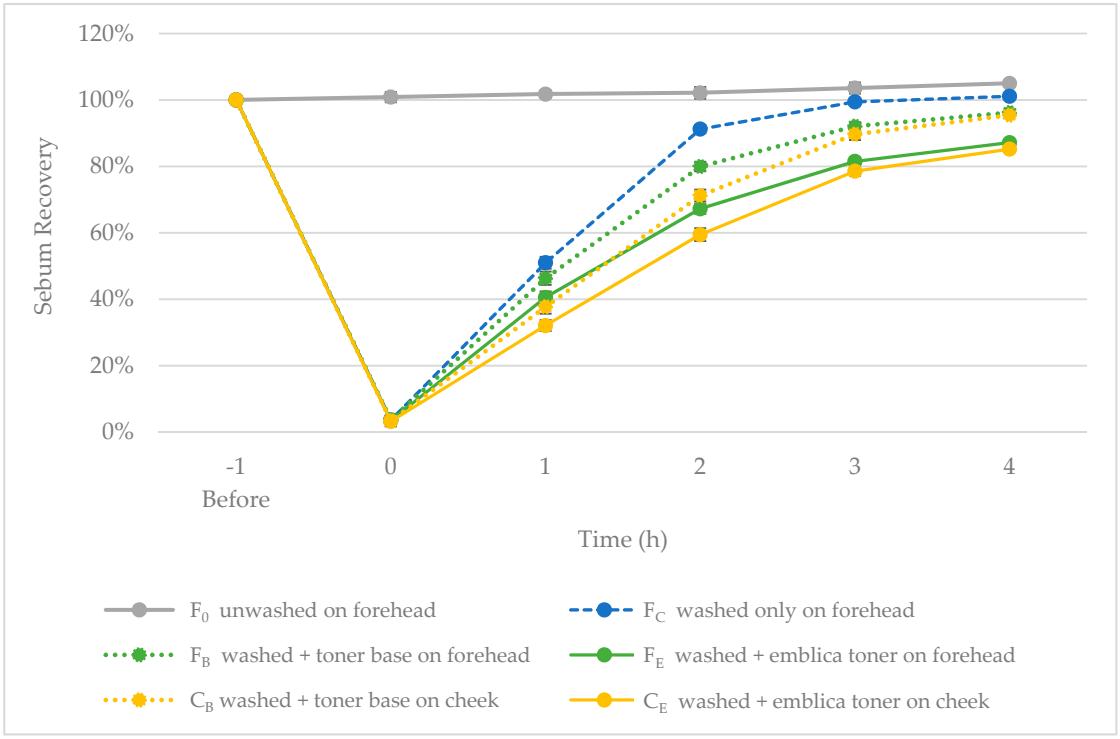
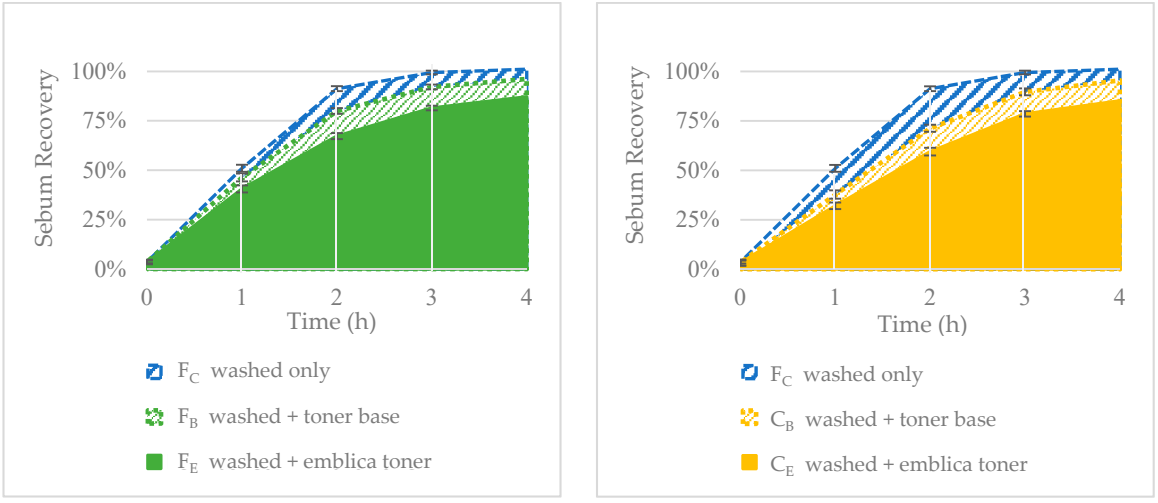


Figure 5. Sebum recovery of various facial skin positions, with designated methods of treatment at different periods, of 30 volunteers.



(**a**) Forehead (**b**) Cheek

Figure 6. Area under the curve (AUC) of sebum recovery of the emblica toner and the toner base on (**a**) forehead and (**b**) cheek skins, at different periods of treatment compared with the control treatment (washed only), of 30 volunteers.

3.6. Anti-Sebum Efficacy

The average anti-sebum efficacy of toner base was found to be 16.4 ± 2.15% at 1 h, and increased to 17.0 ± 1.75% at 2 h, and dropped to 12.4 ± 1.59%, 6.7 ± 1.36% at 3 and 4 h, respectively. The overall anti-sebum efficacy on cheek skin (15.3 ± 2.16%) was higher than that on forehead skin (8.6 ± 1.34%) ($p < 0.05$). The overall anti-sebum efficacy of toner base was found to be 12.0 ± 1.52%.

The average anti-sebum efficacy of emblica toner was found to be 27.2 ± 1.87% at 1 h, and increased to 29.7 ± 1.34% at 2 h, and dropped to 24.6 ± 1.34%, 16.9 ± 1.29% at 3 and 4 h, respectively, higher than that of toner base at 1, 2, 3 and 4 h, and overall ($p < 0.05$). The average anti-sebum efficacy of emblica toner reached its maximum at 2 h. In addition, the overall anti-sebum efficacy on cheek skin (26.9 ± 1.78%) was higher than that on forehead skin (20.1 ± 1.34%) ($p < 0.05$). The overall anti-sebum efficacy of emblica toner was found to be 23.5 ± 1.24%, as shown in Table 3 and Figures 7 and 8.

Table 3. Anti-sebum efficacy of toners on the forehead and cheek skin (F_C, F_B, F_E, C_B, C_E) and the average (E) at 1, 2, 3, and 4 h of 30 volunteers (from Equation (3)).

Time (h)	Anti-Sebum Efficacy (%)					
	Toner Base			Emblica Toner		
	Forehead (F_B)	Cheek (C_B)	Average (B)	Forehead (F_E)	Cheek (C_E)	Average (E)
1	8.3 ± 1.86 ^{AB,a}	24.6 ± 3.07 ^{C,b}	16.4 ± 2.15 ^{FG}	19.0 ± 1.95 $^{\prime\prime IJ,j}$	35.4 ± 3.07 $^{\#L,k}$	27.2 ± 1.87 ^{*OP}
2	11.0 ± 1.69 ^{A,c}	22.9 ± 2.45 ^{C,d}	17.0 ± 1.75 ^{F}	23.8 ± 1.79 $^{\prime\prime K,l}$	35.6 ± 1.87 $^{\#L,m}$	29.7 ± 1.34 ^{*O}
3	9.5 ± 1.51 ^{A,e}	15.2 ± 2.22 ^{D,f}	12.4 ± 1.59 ^{G}	21.8 ± 1.49 $^{\prime\prime IK,n}$	27.4 ± 1.88 $^{\#M,o}$	24.6 ± 1.34 ^{*P}
4	6.0 ± 1.18 ^{B,g}	7.4 ± 1.97 ^{E,g}	6.7 ± 1.36 ^{H}	15.8 ± 1.29 $^{\prime\prime J,p}$	18.1 ± 1.81 $^{\#N,p}$	16.9 ± 1.29 ^{*Q}
Overall	8.6 ± 1.34 ^{h}	15.3 ± 2.16 ^{i}	12.0 ± 1.52	20.1 ± 1.34 $^{\prime\prime q}$	26.9 ± 1.78 $^{\#r}$	23.5 ± 1.24 *

$^{\prime\prime}$ indicates statistically significant differences ($p < 0.05$) between the anti-sebum efficacy of emblica toner and that of toner base in the forehead area in the same row (each period). $^{\#}$ indicates statistically significant differences ($p < 0.05$) between the anti-sebum efficacy of emblica toner and that of toner base in the cheek area in the same row (each period). * indicates statistically significant differences ($p < 0.05$) between the average anti-sebum efficacy of emblica toner and that of toner base in the same row (each period). Different capital letters ($^{A–Q}$) in the same column indicate statistically significant differences ($p < 0.05$) when compared within the same column (between periods of 1, 2, 3 and 4 h). Different lower case letters ($^{a–i}$) in the same row (each period) indicate statistically significant differences ($p < 0.05$) when compared between forehead and cheek position in the toner base column. Different lower case letters ($^{j–r}$) in the same row (each period) indicate statistically significant differences ($p < 0.05$) when compared between forehead and cheek position in the emblica toner column.

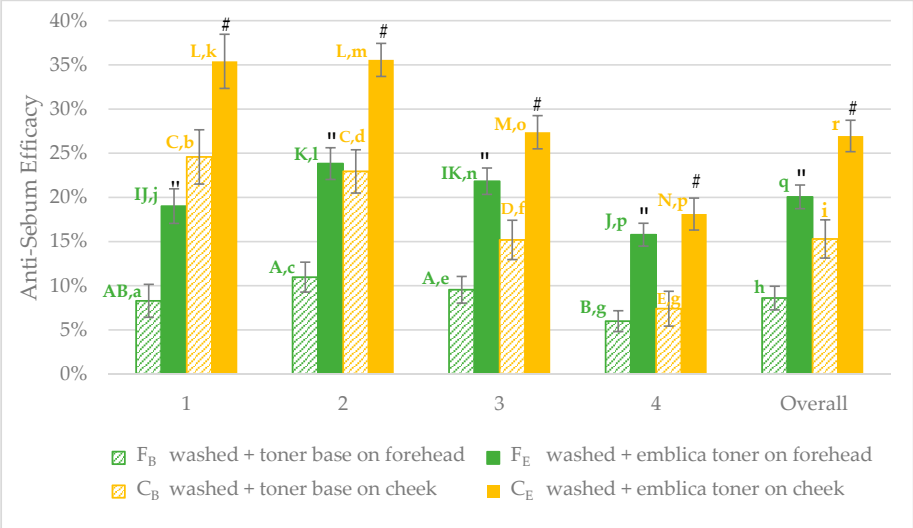


Figure 7. Anti-sebum efficacy of the emblica toners on forehead and cheek skins of treatment compared with the toner base in different periods of 30 volunteers. **″** indicates statistically significant differences between the anti-sebum efficacy of emblica toner and that of toner base in the forehead area in the same period ($p < 0.05$). **#** indicates statistically significant differences between the anti-sebum efficacy of

emblica toner and that of toner base in the cheek area in the same period ($p < 0.05$). Different capital letters ($^{A–E,I–N}$) indicate statistically significant differences ($p < 0.05$) in the same position between periods of 1, 2, 3 and 4 h. Different lower case letters ($^{a–i}$) indicate statistically significant differences ($p < 0.05$) in the same period between forehead and cheek with the toner base applied. Different lower case letters ($^{j–r}$) indicate statistically significant differences ($p < 0.05$) in the same period between forehead and cheek areas with the emblica toner applied.

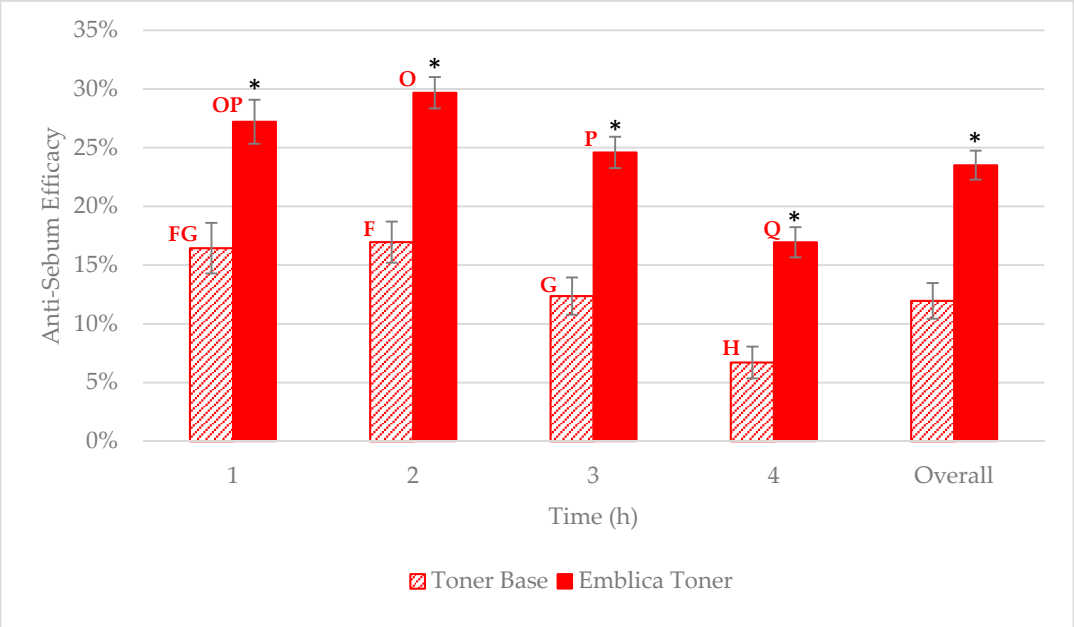


Figure 8. Anti-sebum efficacy of the emblica toners of treatment compared with the toner base at different periods of 30 volunteers. * indicates statistically significant differences between the average anti-sebum efficacy of emblica toner and that of toner base in the same period ($p < 0.05$). Different capital letters (^{F,G,H}) indicate statistically significant differences ($p < 0.05$) in the treatment with the toner base between periods of 1, 2, 3 and 4 h. Different capital letters (^{O,P,Q}) indicate statistically significant differences ($p < 0.05$) in the treatment with the emblica toner between periods of 1, 2, 3 and 4 h.

4. Discussion

4.1. Emblica Extract

Total tannin content of the emblica extract was analyzed to be 40.56 mg tannin/g dried emblica, which was similar to the total tannin content of emblica extract that was extracted with water in a study conducted by Jaijoy et al., which was calculated to be 37.24 mg tannin/g dried emblica [16]. The total tannin content of emblica extract may vary depending upon some factors; for instance, different sources of emblica.

4.2. Development of Emblica Toner

The three formulas of toner base were stable after accelerated stability tests were conducted. With 5-parameter preference test of the toners, it was found that varied glycerin directly influenced the preference test in 10 volunteers, and the results were statistically significantly different in terms of greasiness and absorption ($p < 0.05$), but did not impact the preference test on spreadability, color, and odor since glycerin is a colorless and odorless substance that can adhere the skin and is an effective humectant to conserve stratum corneum hydration [34]; however, if too much glycerin is added, it may become sticky. Because applying toner is a step between facial cleansing and nourishing, toners which have been recently produced are clear, easy to be absorbed, and not sticky, together with

a mild color and odor. Eventually, with all five parameters, toner base formula B with 2% glycerin received the highest score for the preference test.

4.3. Skin Irritation Test

The skin irritation test in 30 volunteers was conducted by a single closed patch test. The three formulas of emblica toners (B1, B2, B3) and the toner base (B) were found to be negative, similar to deionized water (MII = 0 at 1, 24, 48, and 72 h), contrary to the positive control, SLS, (MII = 0.07, 0.18, 0.05, 0 at 1, 24, 48, and 72 h). Moreover, it was found that there were statistically insignificant differences ($p > 0.05$) in the erythema index of different formulas of toners (B, B1, B2, B3) immediately before occlusion and after removal of occlusion at 1, 24, 48, and 72 h. All ingredients are safe and extensively used, and the pH of the toners is suitable for the skin; therefore, the toner base and 1%, 2%, 3% emblica toners were non-irritating to the skin.

4.4. Sebum Level

There were 30 volunteers composed of 16 male and 14 female volunteers in the research. The average sebum casual level, measured by SebumScale®, was 63.14 ± 6.89 μg/cm^2 and the sebum level of forehead skin was statistically significantly higher than sebum level of cheek skin, 66.66 ± 7.01 and 56.12 ± 7.75 μg/cm^2, respectively. These results indicate that the casual sebum level of forehead skin is 1.2 ± 0.11 times of the casual sebum level of cheek skin. Furthermore, the findings showed that forehead skin, which is an area of the T-zone, produces more sebum than cheek skin, which is an area of the U-zone, due to the fact that sebum glands located at the T-zone are denser than sebum glands located at the U-zone, which corresponds to a study conducted by Youn [35].

For the unwashed position (nontreatment), there were statistically insignificant changes in the sebum level ($p > 0.05$). However, there was a trend that was higher than the sebum level before the treatment began (5.0 ± 1.66%). The results indicated that there was homeostasis of the sebaceous gland [36]. For the washed-only position (control treatment), at 0 h, after being washed the sebum levels in all designated positions were statistically insignificantly different ($p > 0.05$), due to the fact that washing the face can reduce the sebum level all over the face. From 0 to 2 h, after being washed, the sebum level recovered constantly with an increasing rate, which corresponded to a study conducted in 100 male and 100 female volunteers in Egypt [37], which revealed that 0.5, 1, and 1.5 h after washing and wiping, the sebum level of forehead skin in both men and women recovered to a steady value. This indicated that sebum recovery trend in the first 1 h and 2 h of different races were not different. From 2 to 3 h after being washed, the sebum level recovered gradually with a reducing rate and reached the casual level at 3 h, corresponding to a study conducted by Rode et al. [33], in which the sebum level on the facial skin reached the casual level after 2–3 h. During 3 to 4 h after being washed, the sebum level recovered gradually with a reducing rate and became steady at the casual level, which still corresponded to the sebum level of the unwashed position (nontreatment) in the same period.

4.5. Sebum Recovery

The sebum recovery changed over time corresponding to the trend of sebum level, and it was found that, at the end of the 4 h experiment, the sebum recovery on the facial skin with either toner base or emblica toner applied was lower than that on the washed-only position. Therefore, it has been shown that the toner base and the emblica toner may reduce the sebum recovery. Moreover, the change was not linear; therefore, for this study, the area under the curve of the sebum recovery was calculated by comparing the area under the curve of the sebum recovery between the skin position that was washed and the toner applied, and the position that was washed only (the control treatment) in the same period and condition, which reduced the impact of confounding factors such as ambient temperature, humidity, daily rhythm, and hormonal balance [32].

4.6. Anti-Sebum Efficacy

For the position with the toner base applied, the anti-sebum efficacy of the toner base was 12.0 ± 1.52%. This showed that applying the toner base after washing can reduce the sebum recovery of the skin more than only washing the face. This might be caused by the fact that washing may remove the lipid film that protects skin hydration which makes sebum glands produce more sebum to compensate the unbalanced hydration. As a result, washing without the toners stimulates the skin to secrete more sebum than the skin which is washed before the toner base is applied, which is composed of glycerin, one of the effective humectants that can increase skin hydration, which compensates moisture to the skin, corresponding to the results of study with a 4-h experiment, the same period as this study, which revealed that skincare products that moisten the skin can reduce sebum secretion [38].

The anti-sebum efficacy of the emblica toner was 23.5 ± 1.24%, higher than that of the toner base ($p < 0.05$) and gave the highest anti-sebum efficacy at 2 h. The anti-sebum efficacy on cheek skin (26.9 ± 1.78%) was higher than that on forehead skin (20.1 ± 1.34%). It showed that the astringent property of emblica extract in emblica toner can reduce sebum more than toner base, corresponding to a study conducted by Hofmann et al. [22] which suggested that tannin can form cross-linked bonds with protein, which may result in the astringent effect to the skin in reducing sebum secretion. In addition, applying equal amounts of emblica toner in each position gave less anti-sebum efficacy on forehead skin than that on cheek skin since forehead skin secreted more sebum than cheek skin. The average anti-sebum efficacy of 3% emblica toner in this study was relatively high compared with several studies. Typically, anti-sebum efficacy of more than 10% produced a satisfying result [39]. For a toner with 6% guava leaf extract, the anti-sebum efficacy of the forehead and nose was found to be 13.10 ± 3.67% and 21.43 ± 3.21%, respectively, after 28-day usage [24]. For a toner with 2%, 4.5%, and 7% green tea extract, the anti-sebum efficacy was found to be 3.47 ± 0.10%, 8.18 ± 0.44%, and 17.87 ± 0.46%, respectively, after 14-day usage, and 8.48 ± 0.13%, 20.26 ± 1.03%, and 31.57 ± 1.22%, respectively, after 28-day usage [25].

Applying emblica toner on forehead skin and cheek skin after washing resulted in anti-sebum efficacy of facial skin under testing conditions around the 4-h period of the experiment. The anti-sebum efficacy on cheek skin was higher than the anti-sebum efficacy on forehead skin. In addition, the efficacy reached its maximum at 2 h after applying emblica toner, and was statistically significantly higher than the efficacy of toner base throughout the period of the experiment. Moreover, the model of anti-sebum evaluation used in this research can be applied for evaluation of other anti-sebum products for further studies.

5. Conclusions

This study has indicated that the emblica toner is safe and has anti-sebum efficacy on facial skin. Moreover, the model of anti-sebum efficacy evaluation applied in this study, adding nontreatment (unwashed skin position) and control treatment (only-washed skin position) to the middle of the forehead using a split-face method, makes it possible to compare the sebum levels at the same time and reduce the impact of confounding factors, and then calculate the anti-sebum efficacy on the facial skin from the area under the curve of the sebum recovery on each designated position with different treatments. As a result, this research may give an overview of study of anti-sebum efficacy on facial skin under controlled conditions in order to be useful for further study.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Erythema index of skin Irritation test.

Reagent	Erythema Index				
	Before Occlusion	After Removal of the 24-h Occlusion Patches			
		1 h	24 h	48 h	72 h
DI Water	438.07 ± 3.40	436.60 ± 3.40	435.24 ± 3.02	437.49 ± 3.64	437.31 ± 3.58
SLS	438.08 ± 3.55	465.06 ± 3.55 *	480.93 ± 3.53 *	452.12 ± 3.09 *	439.68 ± 3.39
B	438.83 ± 2.89	436.12 ± 3.29	436.88 ± 3.58	435.11 ± 3.15	435.80 ± 3.13
B1	436.26 ± 3.40	432.26 ± 3.30	433.65 ± 3.45	433.83 ± 3.33	435.71 ± 3.36
B2	434.60 ± 2.97	431.42 ± 2.93	431.27 ± 3.04	433.39 ± 2.88	434.63 ± 2.77
B3	437.96 ± 2.73	432.63 ± 3.29	434.77 ± 3.14	435.68 ± 3.08	437.15 ± 3.23

* indicates statistically significant differences between erythema index before occlusion and after removal of 24-h occlusion patches ($p < 0.05$) for the same reagent (DI water = Deionized water (negative control), SLS = Sodium lauryl sulfate (positive control), B = Toner base, B1 = Toner base with 1% emblica extract, B2 = Toner base with 2% emblica extract, B3 = Toner base with 3% emblica extract).

Table A2. The sebum recovery on the forehead and cheek skin (F_C, F_B, F_E, C_B, C_E) at 1, 2, 3, and 4 h of 30 volunteers (from Equation (1)).

Time (h)	The Sebum Recovery (%)					
	Unwashed (Nontreatment)	Washed Only (Control Treatment)	Washed			
			Toner Base		Emblica Toner	
	Forehead (F_0)	Forehead (F_C)	Forehead (F_B)	Cheek (C_B)	Forehead (F_E)	Cheek (C_E)
−1	100.0	100.0	100.0	100.0	100.0	100.0
0	100.9 ± 1.24	3.6 ± 0.28	3.7 ± 0.31	3.4 ± 0.30	3.5 ± 0.29	3.2 ± 0.28
1	101.8 ± 1.56	51.0 ± 0.72	46.3 ± 1.04	37.7 ± 1.55	40.5 ± 1.09	32.1 ± 1.48
2	102.1 ± 1.13	91.3 ± 1.77	80.0 ± 2.01	71.2 ± 2.10	67.3 ± 1.79	59.5 ± 1.66
3	103.6 ± 1.75	99.4 ± 1.23	92.1 ± 1.43	89.7 ± 1.76	81.5 ± 1.51	78.6 ± 1.93
4	105.0 ± 1.66	101.1 ± 0.95	96.2 ± 1.20	95.5 ± 1.73	87.2 ± 1.17	85.2 ± 1.37

Table A3. Area under the curve (AUC) of sebum recovery on the forehead and cheek skin (F_C, F_B, F_E, C_B, C_E) at 1, 2, 3, and 4 h of 30 volunteers.

Time (h)	Area under the Curve of Sebum Recovery vs. Time (%·h)				
	Washed Only (Control Treatment)	Washed			
		Toner Base		Emblica Toner	
	Forehead (F_C)	Forehead (F_B)	Cheek (C_B)	Forehead (F_E)	Cheek (C_E)
1	27.3 ± 0.42	25.0 ± 0.61	20.6 ± 0.87	22.0 ± 0.56	17.6 ± 0.85
2	71.1 ± 1.11	63.1 ± 1.37	54.5 ± 1.69	53.9 ± 1.19	45.8 ± 1.45
3	95.3 ± 1.28	86.0 ± 1.55	80.5 ± 1.89	74.4 ± 1.54	69.0 ± 1.73
4	100.3 ± 0.96	94.2 ± 1.30	92.6 ± 1.71	84.3 ± 1.27	81.9 ± 1.62
Overall	294.0 ± 3.43	268.4 ± 4.47	248.1 ± 5.82	234.6 ± 4.15	214.3 ± 5.19

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