



Research Article

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Anti-Aging Efficacy of *Averrhoa bilimbi* Fruit Extract Cream: A Human Clinical Trial

[Efektivitas Krim Ekstrak Buah *Averrhoa bilimbi* terhadap Parameter Penuaan Kulit: Studi Uji Klinis pada Manusia]

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ABSTRACT

Averrhoa bilimbi, a plant native to Indonesian, exhibits antioxidant, sunscreen, and tyrosinase inhibitory activities due to its polyphenol and flavonoid content. This study aimed to evaluate the anti-aging efficacy of a topical cream containing *A. bilimbi* fruit extract through a human clinical trial. The extract was obtained by maceration using 70% ethanol and formulated into a water-in-oil (W/O) cream at concentrations of 1% (F1), 3% (F2), and 5% (F3). Each formulation was evaluated for its physical characteristics, including organoleptic properties, homogeneity, pH, and viscosity. Irritation tests were conducted on animals and human volunteers. A total of 24 human subjects applied the cream for 30 days. Skin condition parameters—sebum, moisture, pigmentation, pore size, elasticity, and collagen fibers—were assessed before and after treatment using a skin analyzer. The results showed that the F3 formulation produced the most notable improvements, including increased skin moisture and elasticity, and decreased pigmentation and pore size. Statistical analysis revealed significant improvements ($p < 0.05$) in pore size and elasticity for the F3 group. These findings indicate that the 5% *A. bilimbi* extract cream is a safe and effective anti-aging topical formulation.

ABSTRAK

Belimbing wuluh (*Averrhoa bilimbi*) merupakan tanaman asli Indonesia yang mengandung senyawa aktif seperti polifenol dan flavonoid, serta memiliki aktivitas antioksidan, tabir surya, dan penghambat enzim tirosinase. Penelitian ini bertujuan mengevaluasi efektivitas krim yang mengandung ekstrak buah *A. bilimbi* dalam mengatasi tanda-tanda penuaan kulit melalui uji klinis pada manusia. Ekstrak buah diperoleh melalui maserasi dengan etanol 70% dan diformulasikan menjadi krim tipe air dalam minyak (A/M) dengan konsentrasi 1% (F1), 3% (F2), dan 5% (F3). Evaluasi karakteristik sediaan meliputi uji organoleptik, homogenitas, pH, dan viskositas. Uji iritasi dilakukan pada hewan dan manusia. Sebanyak 24 subjek relawan menggunakan krim selama 30 hari. Evaluasi parameter kulit dilakukan sebelum dan sesudah penggunaan dengan bantuan *skin analyzer*, yang mencakup kadar sebum, kelembaban, pigmentasi, diameter pori, elastisitas, dan serat kolagen. Hasil menunjukkan bahwa formula F3 memberikan hasil paling optimal, dengan peningkatan kelembaban dan elastisitas kulit, serta penurunan pigmentasi dan diameter pori secara bermakna ($p < 0,05$). Oleh karena itu, krim ekstrak buah *A. bilimbi* konsentrasi 5% berpotensi sebagai sediaan antipenuaan yang aman dan efektif.



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1. INTRODUCTION

Skin aging is a complex biological process, with approximately 80% of visible signs attributed to ultraviolet (UV) exposure, a condition commonly known as photoaging. This process primarily affects sun-exposed areas such as the face, neck, hands, and lower limbs (Aramo, 2012). UVB radiation primarily damages the epidermis, whereas UVA rays penetrate deeper into the dermis, leading to the formation of reactive oxygen species (ROS) and free radicals that degrade essential structural proteins, including collagen and elastin (Binic et al., 2013).

In individuals with Asian skin types, aging is often marked by reduced sebum production, increased pigmentation, formation of wrinkles, and a decline in moisture retention (Dayan, 2008). Topical cosmetic products containing natural bioactive compounds are increasingly preferred due to their dual function of preventing skin aging and maintaining skin health. Phytochemicals such as flavonoids, phenolic acids, saponins, and alkaloids are known to stimulate collagen synthesis and exhibit antioxidant properties, making them particularly suitable for the formulation of anti-aging skin products (Suharsanti et al., 2019).

Averrhoa bilimbi, a tropical plant native to Indonesia, has demonstrated significant potential in skin care applications. Its fruit contains notably higher levels of phenolic and flavonoid compounds compared to its leaves, with total phenolic content reported at 19.80 ± 1.67 mg GAE/g and total flavonoid content at 24.75 ± 0.33 mg RE/g (Suharsanti et al., 2019). Furthermore, the fruit extract has been shown to possess a strong sun protection factor (SPF) at concentrations of 300 ppm, antioxidant activity with an IC_{50} of 2.33 ± 0.33 mg/mL, and tyrosinase inhibition with an IC_{50} of 186.85 ± 9.37 mg/mL.

Previous research on *A. bilimbi* has largely focused on its wound-healing capabilities, particularly using leaf extracts. However, the potential of the fruit extract for topical anti-aging applications remains underexplored. Creams are widely favored as topical delivery systems due to their ease of application, aesthetic appeal, and skin compatibility.

Given these considerations, the present study aimed to evaluate the anti-aging effects of a cream formulation containing *A. bilimbi* fruit extract through a clinical trial involving human participants. The efficacy was assessed based on key skin parameters, including moisture, pigmentation, elasticity, pore size, sebum level, and collagen fiber density.

2. METHOD

2.1. Materials

Dried *A. bilimbi* fruits were used as the plant material. Other ingredients included ethanol 70% (Bratachem, Indonesia), triethanolamine (TEA), glycerin, stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor,

Indonesia). Experimental animals were male New Zealand rabbits aged 12–16 weeks. Human subjects were healthy male and female volunteers aged 19–25 years. Equipment included a pH meter (Hanna Instruments, Indonesia), a viscometer (Brookfield, USA), and a skin analyzer (Rista Clinic Beauty Care, Demak Regency, Central Java, Indonesia).

2.2. Extraction of *A. bilimbi*

A total of 200 g of dried powdered *A. bilimbi* fruit was extracted by maceration with 70% ethanol for 3 days at room temperature. The extract was then concentrated using a rotary evaporator at 60°C and 100 rpm to obtain a viscous crude extract (Rahardhian et al., 2019).

2.3. Cream Formulation

Creams were prepared with *A. bilimbi* extract concentrations of 1% (F1), 3% (F2), and 5% (F3) (Table 1), following the method described by Iskandar et al. (2016). The water-soluble phase—consisting of TEA, nipagin, and glycerin in distilled water—was heated to 70°C. Simultaneously, the oil phase—comprising stearic acid, cetyl alcohol, stearyl alcohol, and nipasol—was heated to the same temperature. The aqueous phase was gradually added to the oil phase under continuous stirring to form a stable emulsion. After the emulsion cooled slightly, the extract was incorporated and mixed thoroughly until homogeneous.

Table 1. Composition of *A. bilimbi* Fruit Extract Cream Formulations

Ingredient	F1 (%)	F2 (%)	F3 (%)
<i>A. bilimbi</i> extract	1	3	5
Stearic Acid	24	24	24
Cetyl Alcohol	4	4	4
Stearyl alcohol	2	2	2
Nipasol	0.5	0.5	0.5
Nipagin	0.5	0.5	0.5
Glycerin	28	28	28
TEA	2	2	2
Distilled water	ad 100	ad 100	ad 100

Note: “ad 100” indicates addition of distilled water to make the total quantity 100%

2.4. Ethical Clearance

The study received ethical approval from the Bioethics Commission for Medical and Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang (Approval No. 313/V/2019/Bioethics Commission). This clearance encompassed both animal and human testing.

2.5. Irritation Test

Irritation tests were conducted in compliance with OECD guidelines (OECD, 2021). A 6 cm² shaved dorsal skin area of each rabbit was treated with 0.5 g of cream under a semi-occlusive bandage. Observations for erythema and edema were recorded at

1, 24, 48, and 72 hours, using the Draize scoring method (Table 2). The same protocol was applied to human volunteers, with additional daily monitoring during the first three days of use.

Table 2. Irritation Score (Draize Scoring System)

Condition	Score
There isn't any	0
Very Light	1
Clearly Visible	2
Heavy	3
Very heavy	4

2.6. Informed Consent

All human volunteers signed informed consent forms, confirming they understood the study objectives and procedures, and agreed to participate voluntarily. Inclusion criteria included healthy male or female participants aged 19–25 years, with no history of chronic illness, skin disease, supplement intake, or current medication. Exclusion criteria included degenerative or inflammatory conditions, pregnancy, and breastfeeding.

2.7. Human Trial

Twenty-four volunteers were assigned to three groups and instructed to apply the cream twice daily (morning and evening) to the dorsal area of one hand for 30 days. Skin conditions were assessed on day 1 and day 30 using a professional skin analyzer operated by a physician. Parameters evaluated included sebum, moisture, pigmentation, pore size, elasticity, and collagen fiber density. Daily self-assessments were also recorded using a portable skin analyzer to monitor adherence and condition changes.

2.8. Analysis Data

The data obtained from skin analysis before and after the 30-day treatment were analyzed using paired t-tests for each group. A p-value of < 0.05 was considered statistically significant. The formulation yielding the most statistically significant improvement was considered the most effective.

3. RESULTS AND DISCUSSION

3.1. Characteristics of The Anti-Aging Cream

The formulated anti-aging creams (F1, F2, and F3) appeared semi-solid with a characteristic fruity odor and color ranging from light yellow to deep yellow depending on the extract concentration. All formulations were physically homogeneous. Detailed organoleptic and homogeneity observations are summarized in Table 3.

The pH values of all formulations increased slightly with increasing extract concentration but remained within the acceptable topical range of 4.5–6.5, indicating compatibility with skin physiology (Figure 1A). Viscosity was also observed to increase with extract concentration but remained within the quality standard of 2,000–50,000 cPs for cream preparations (Figure 1B), complying with SNI 16-4399-1996. Optimal viscosity enhances cream spreadability and skin absorption.

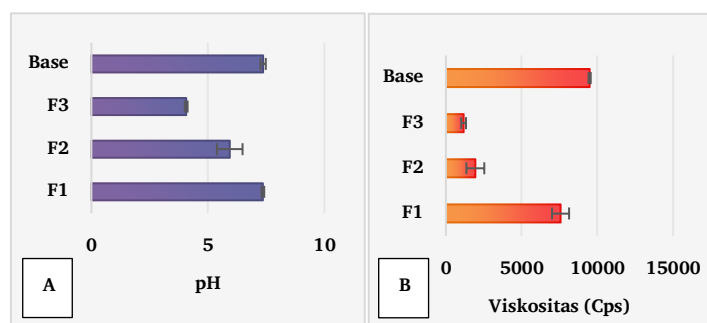


Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

Table 3. Organoleptic and Homogeneity Characteristics of Anti-aging Cream

Formula	Smell	Form	Color	Homogeneity
Base	<i>A. bilimbi</i> fruit	Semi solid	White	Homogeneous
F1	<i>A. bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
F2	<i>A. bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
F3	<i>A. bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

3.2. Irritation Test Results

No visible signs of erythema or edema were observed in either rabbit or human subjects after 72 hours of application. Based on Draize scoring (Table 4), all formulations—including the base and F1–F3—scored 0, indicating they were non-irritating.

Table 4. Irritation Scores of Anti-aging Creams on Animals and Humans

Formula	Animal Irritation Index	Human Irritation Index
Base	0	0
F1	0	0

F2	0	0
F3	0	0

3.3. Human Trial: Anti-Aging Parameters

Skin condition parameters before and after 30 days of application were evaluated. The skin condition improvements are visualized in Figure 2. The F3 formulation (5% *A. bilimbi* extract) consistently exhibited the most pronounced improvements. Sebum levels showed a slight increase in both F2 and F3 groups, possibly due to the oil-rich water-in-oil (W/O) base; however, the changes were not statistically significant ($p > 0.05$). Skin hydration improved in all groups, with F3 achieving a 22.34% increase, although this was also not statistically significant ($p > 0.05$). The

moisturizing effect may be attributed to the presence of flavonoids, but a longer treatment duration may be necessary to observe significant outcomes (Rasul & Akhtar, 2012).

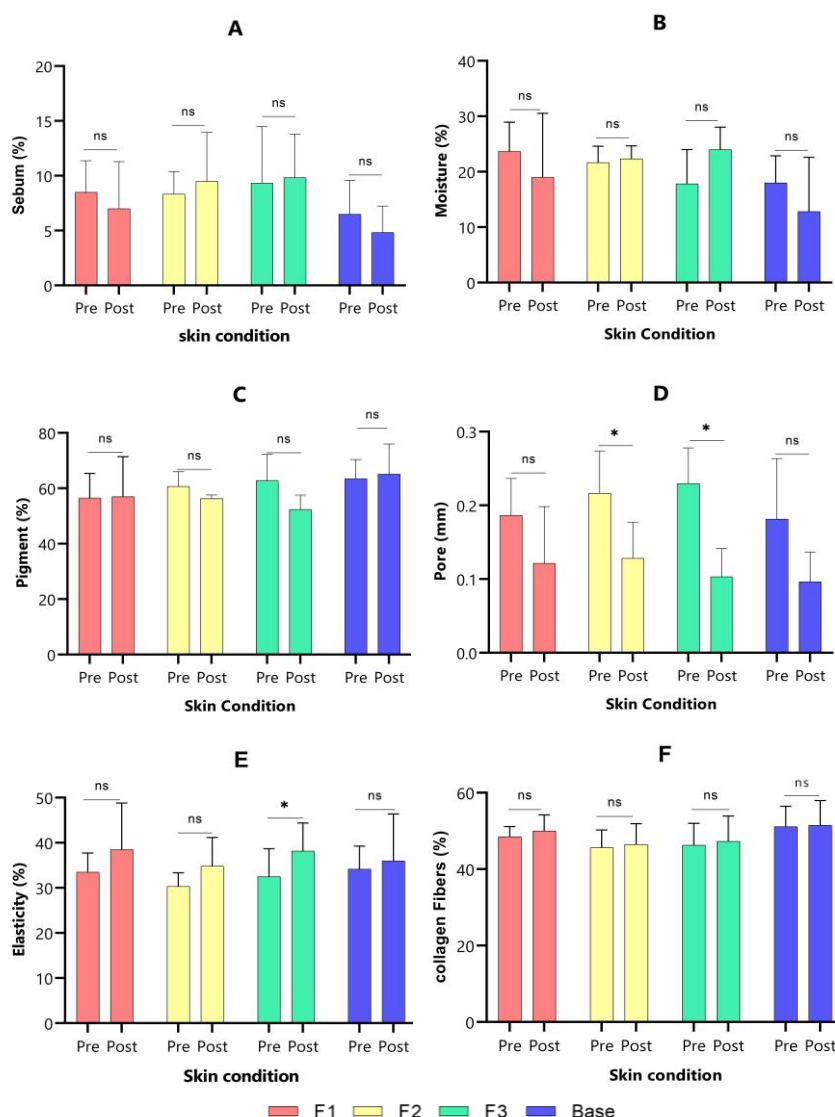


Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers, Mean ± SD (n=6), ns = no significant, * = significant (p<0,05) with t test

Pigmentation was reduced by 15.08% in the F3 group, although the decrease did not reach statistical significance ($p > 0.05$). This finding aligns with previous research suggesting that visible depigmentation generally requires longer application periods (Khan et al., 2014). Pore size reduction was statistically significant ($p < 0.05$) in both F2 and F3 groups, with F3 demonstrating the most notable effect—a 54.18% decrease. This result is likely due to the astringent properties of phenolic compounds, including tannins (Puspitasari, 2011).

A significant increase in skin elasticity was observed in the F3 group (16.85%, $p < 0.05$), indicating that the extract may contribute to the preservation or stimulation of collagen, which in turn improves dermal structure and function (Jadoon et al., 2015). Although the collagen fiber density also showed a slight improvement (1.12%) in the F3 group, it was not statistically significant, suggesting that a longer usage period or a higher

extract concentration might be required for measurable collagen enhancement.

The F3 formulation demonstrated statistically significant improvements in pore size and elasticity, while other parameters showed positive trends. These findings support the use of *A. bilimbi* extract in topical anti-aging formulations and suggest that longer treatment periods may yield more pronounced effects.

CONCLUSION

Based on the findings from this study, the cream formulation containing 5% *A. bilimbi* extract (F3) demonstrated the most significant anti-aging effects, particularly in reducing skin pore diameter and improving skin elasticity, both of which were statistically significant ($p < 0.05$). Although the changes in sebum production, moisture content, pigmentation, and collagen fiber density did not reach statistical significance, positive trends were observed. These findings indicate that *A. bilimbi* fruit extract holds

promising potential as a safe and effective active ingredient in topical anti-aging skincare products. Further studies with longer application durations and potentially higher extract concentrations are recommended to confirm and extend these findings.

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