BUKTI KORESPONDENSI
JURNAL NASIONAL TERAKREDITASI PERINGKAT 2

Judul artikel	:	Anti-Aging Efficacy of Averrhoa bilimbi Fruit Extract Cream: A Human Clinical Trial
Jurnal	:	Jurnal Jamu Indonesia (JJI)
Edisi	:	Tahun 2025, Vol 10 No 2, hal 116–120
Penerbit	:	Institut Pertanian Bogor PUI-PT TropBRC
Penulis	:	Ririn Suharsanti, Muhammad Ryan Radix Rahadhian, Nining Sugihartini, Endang Lukitaningsih

Tabel Tahapan Publikasi Artikel

No	Tahapan Publikasi	Tanggal	Halaman
1	Submission of manuscript	10 Mei 2025	2
2	Editorial Decision : Send to Review	10 Mei 2025	3
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11	Article published Vol. 10 No. 2 (2025) https://jamu-journal.ipb.ac.id/index.php/JJI/article/view/401/202	26 Mei 2025	12
12	Lampiran bukti korespondensi	-	13-114

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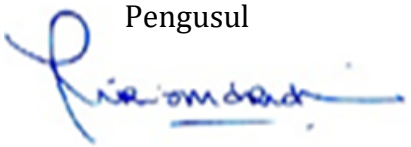

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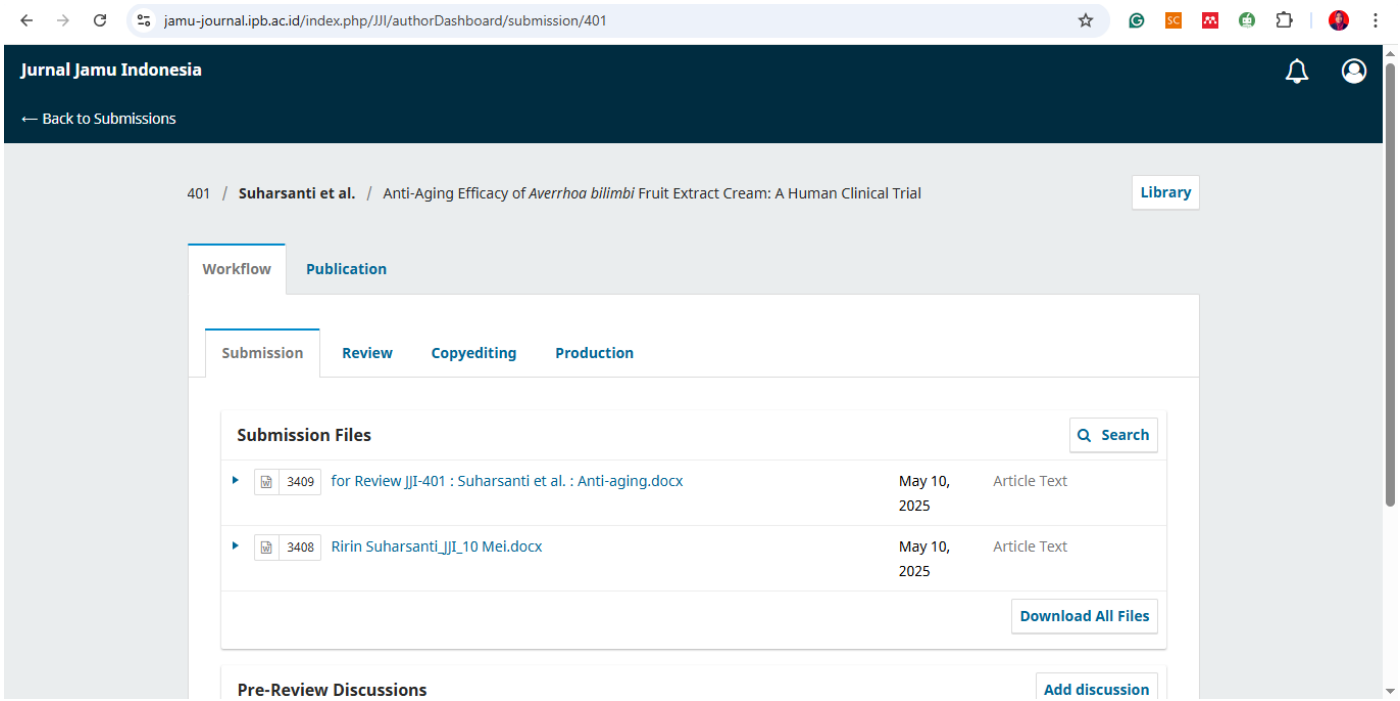

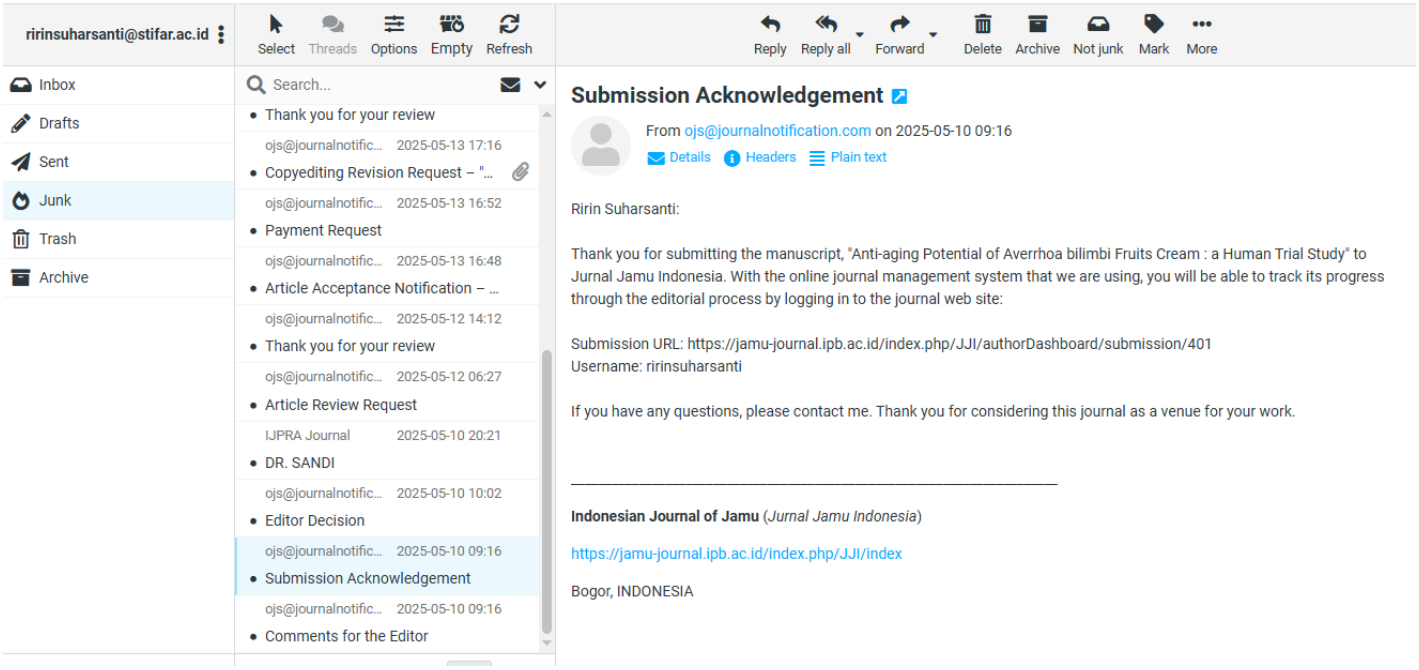

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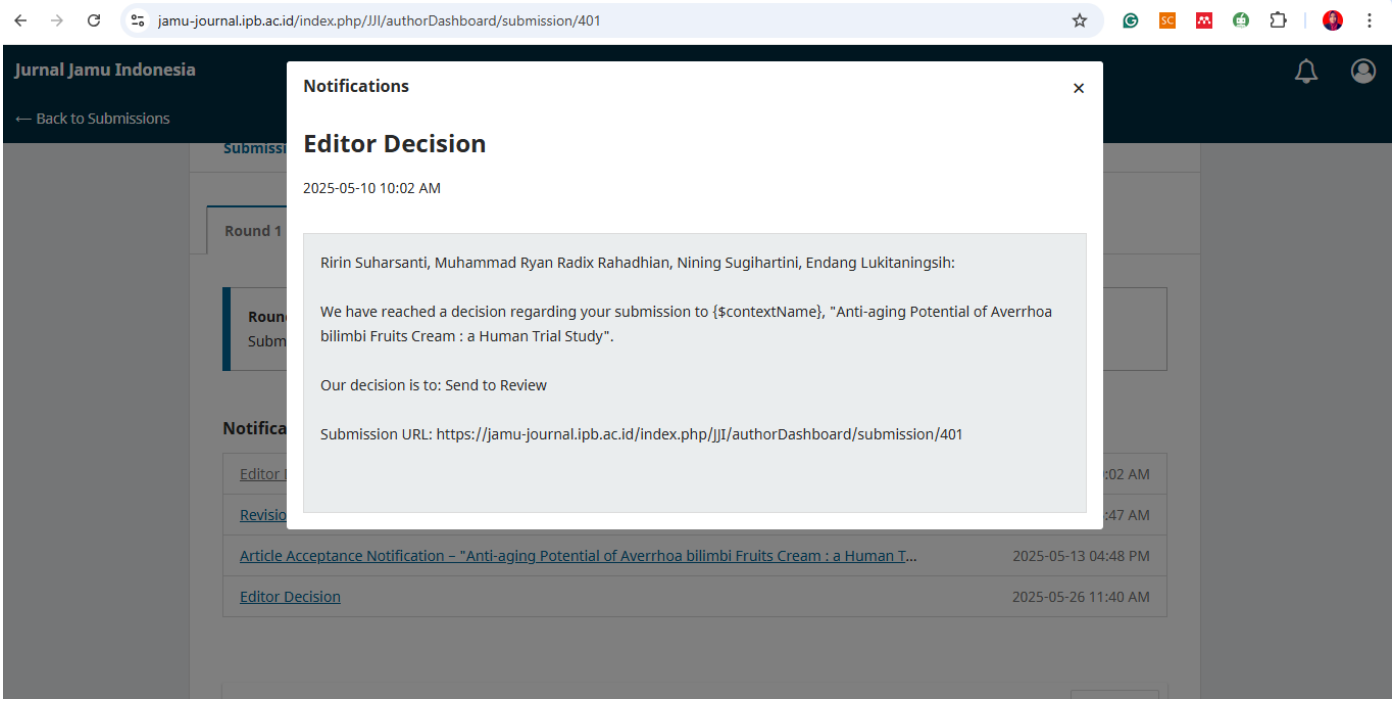

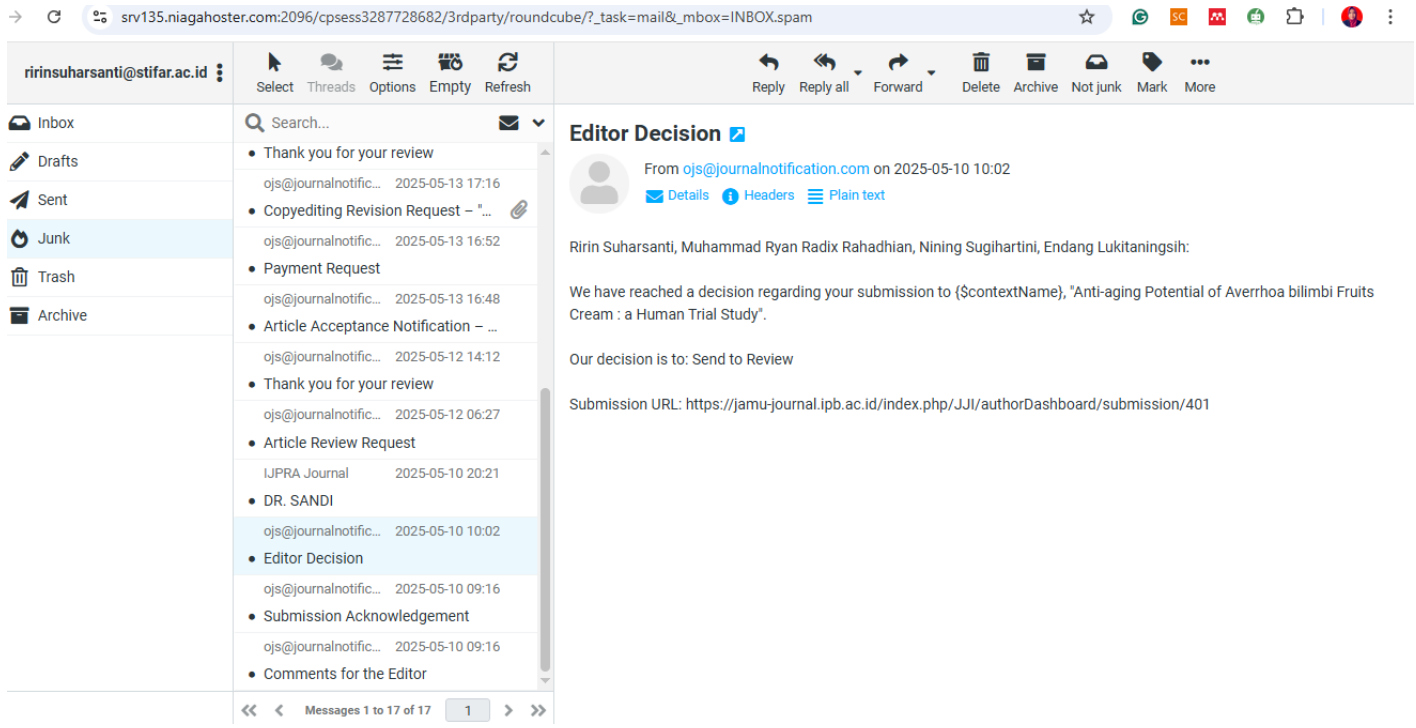

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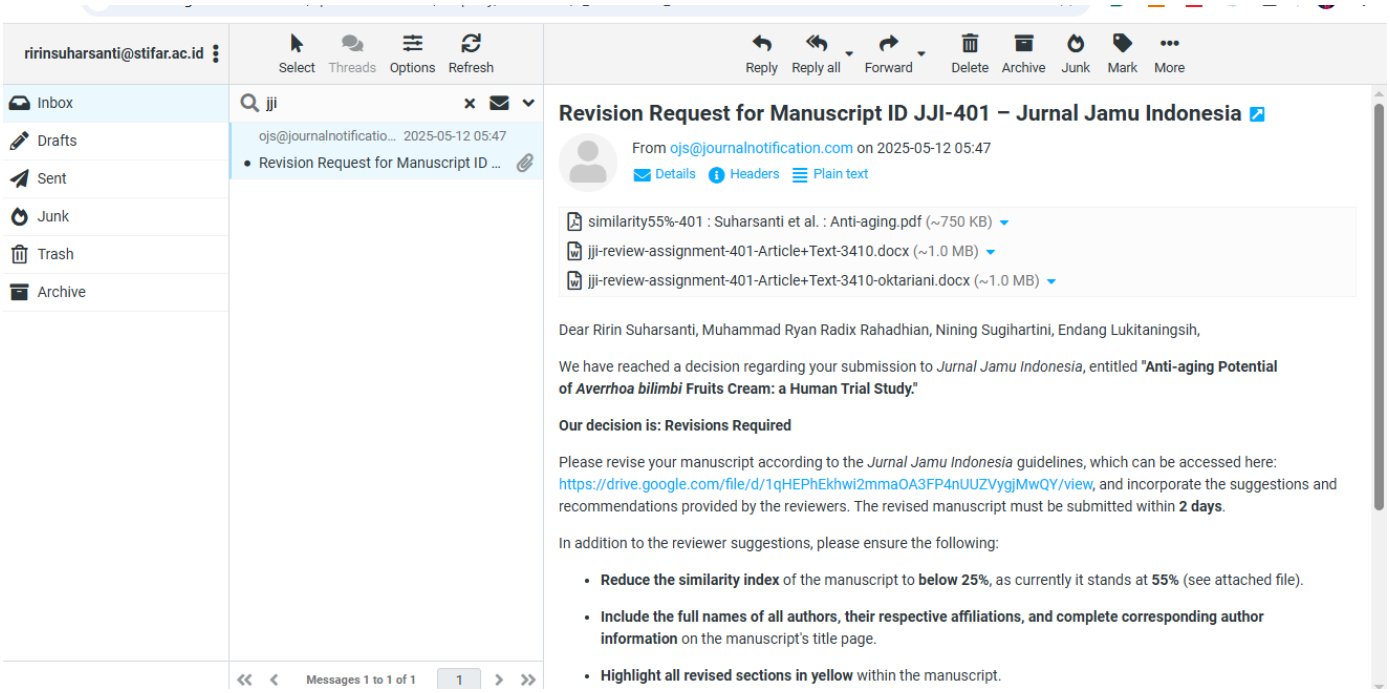

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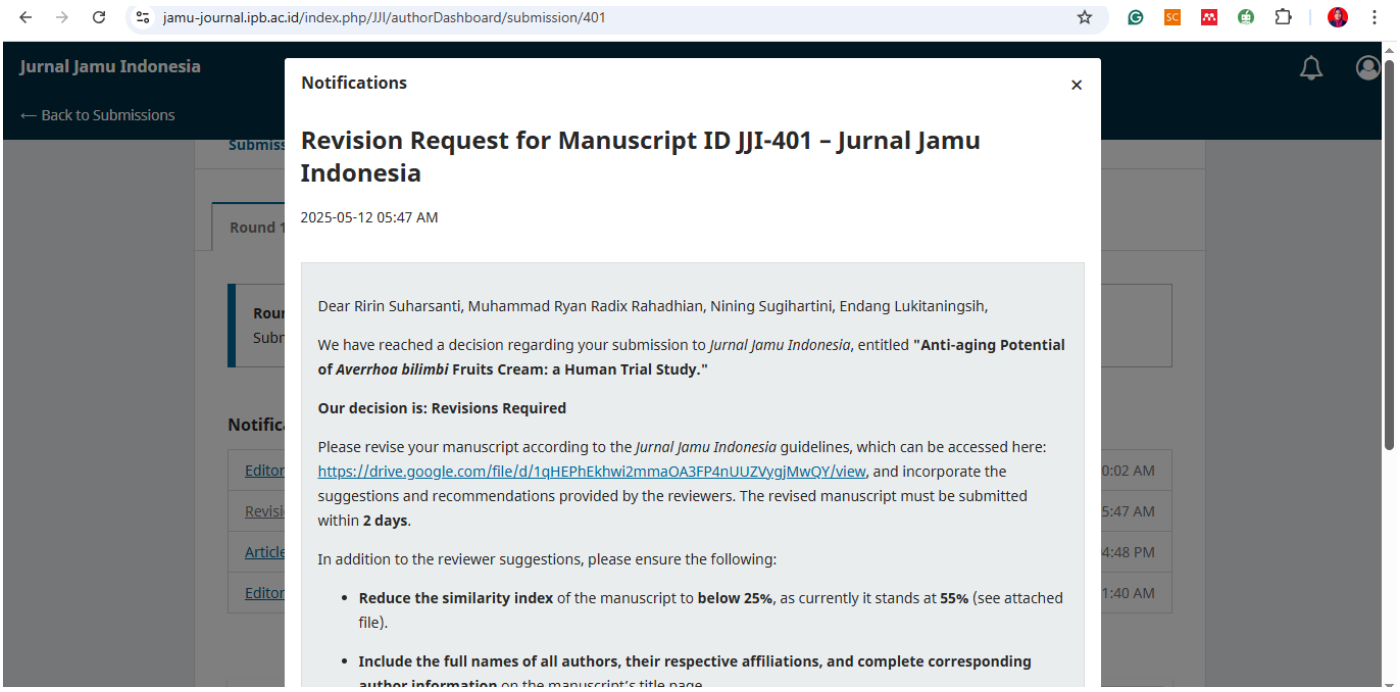

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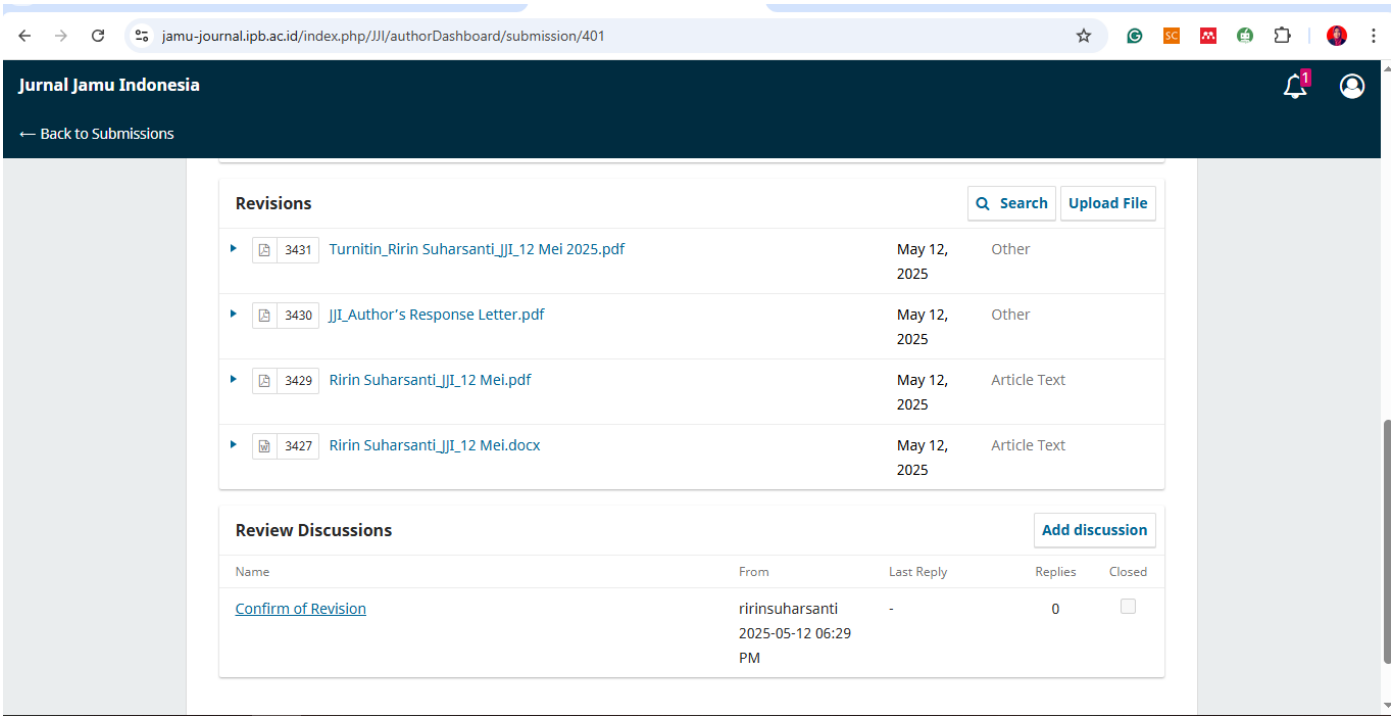

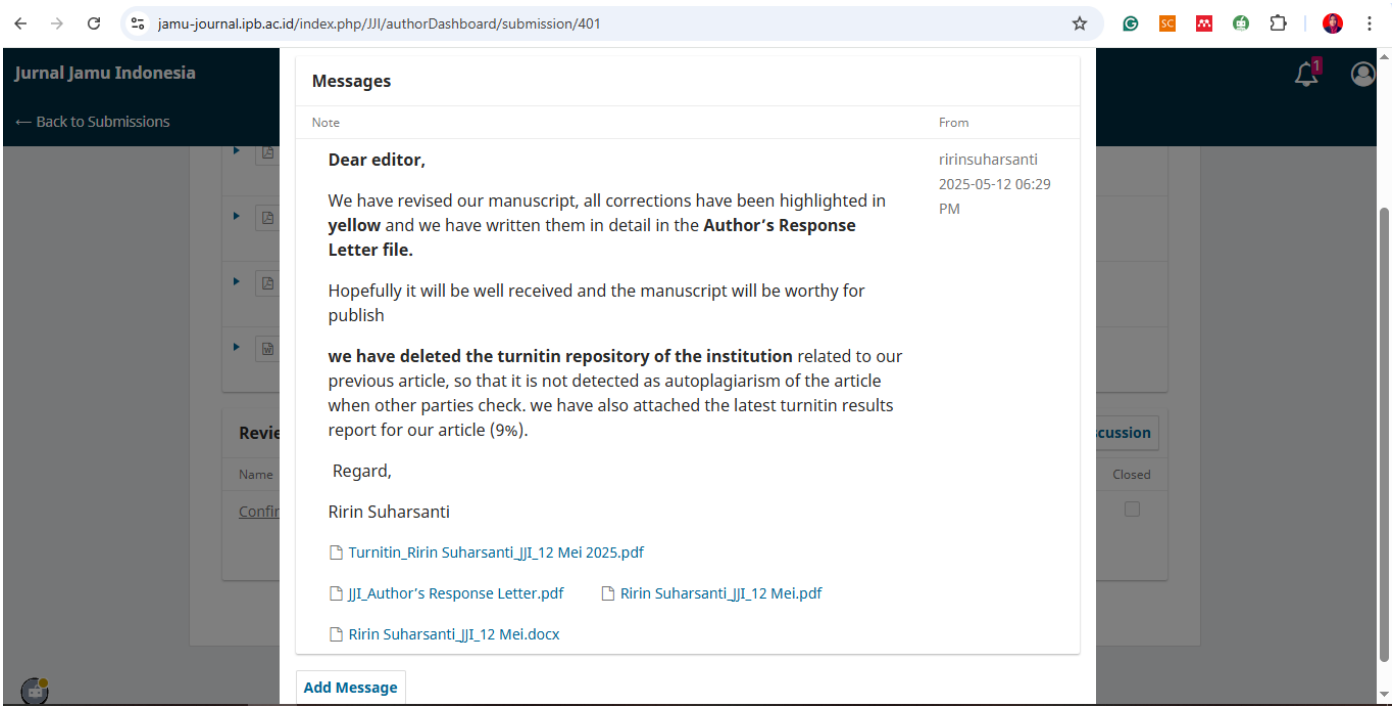

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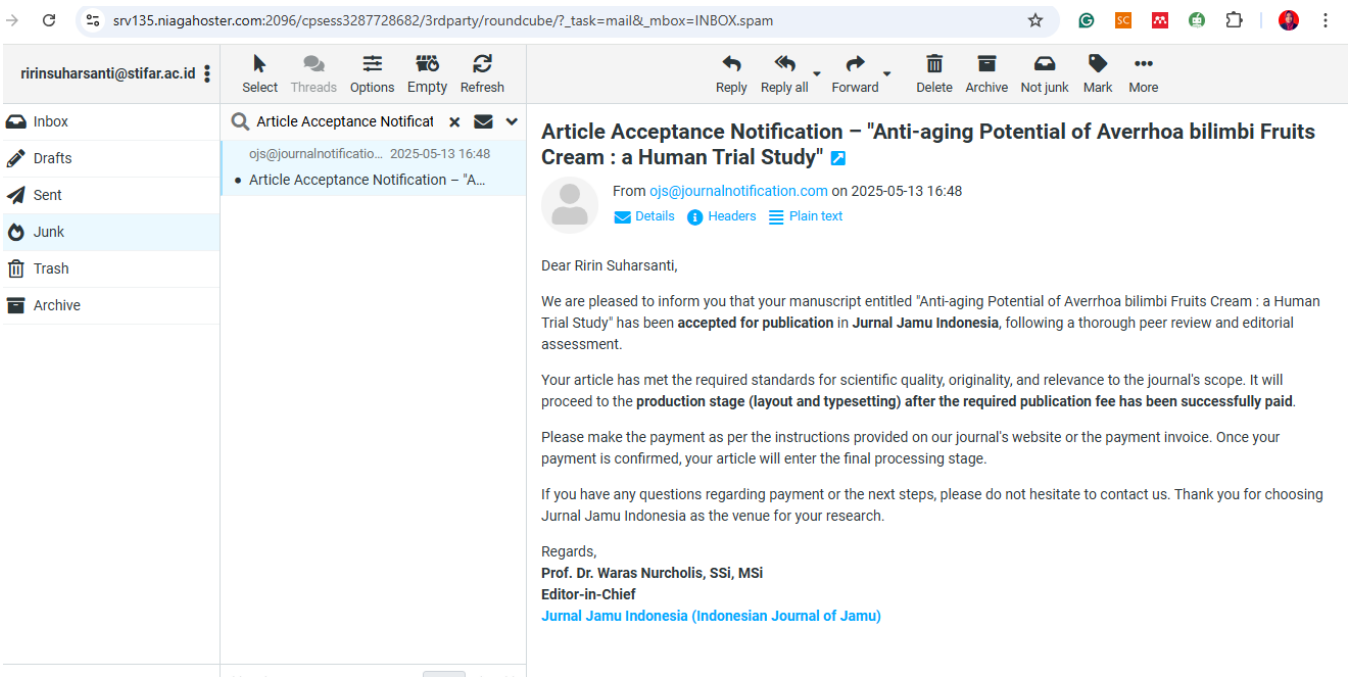

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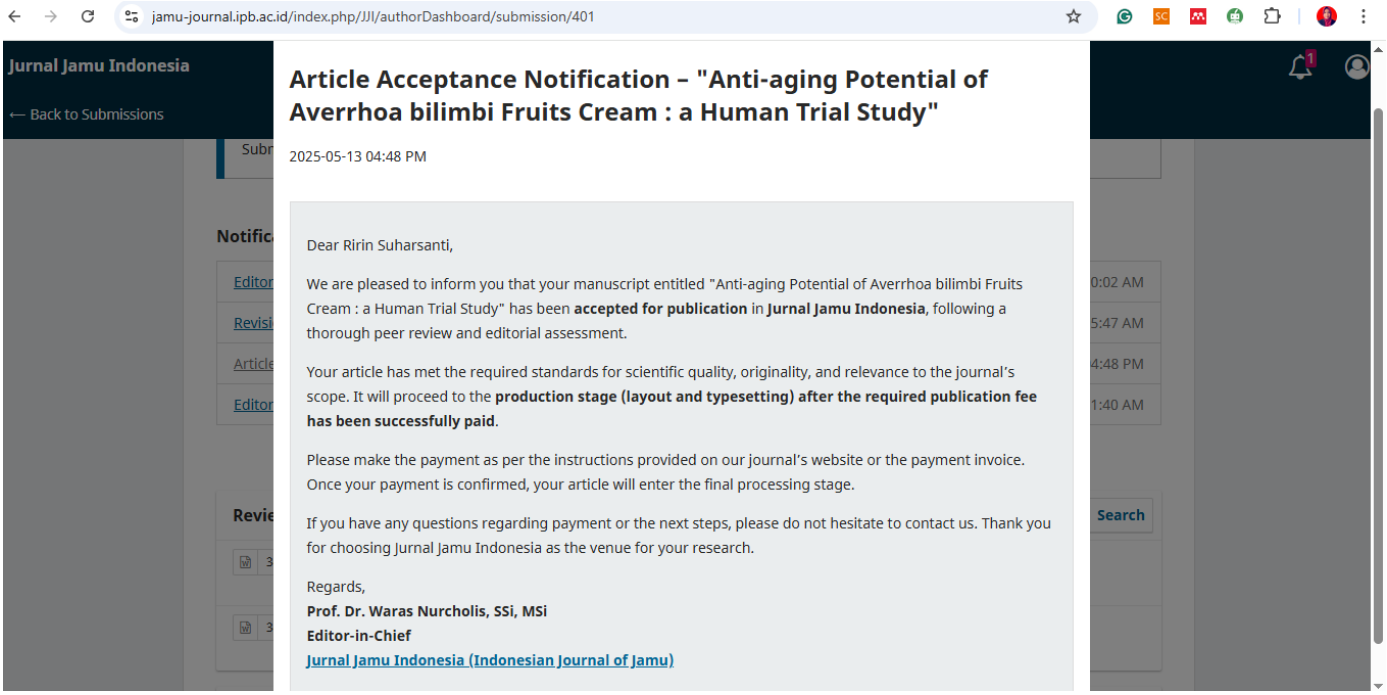

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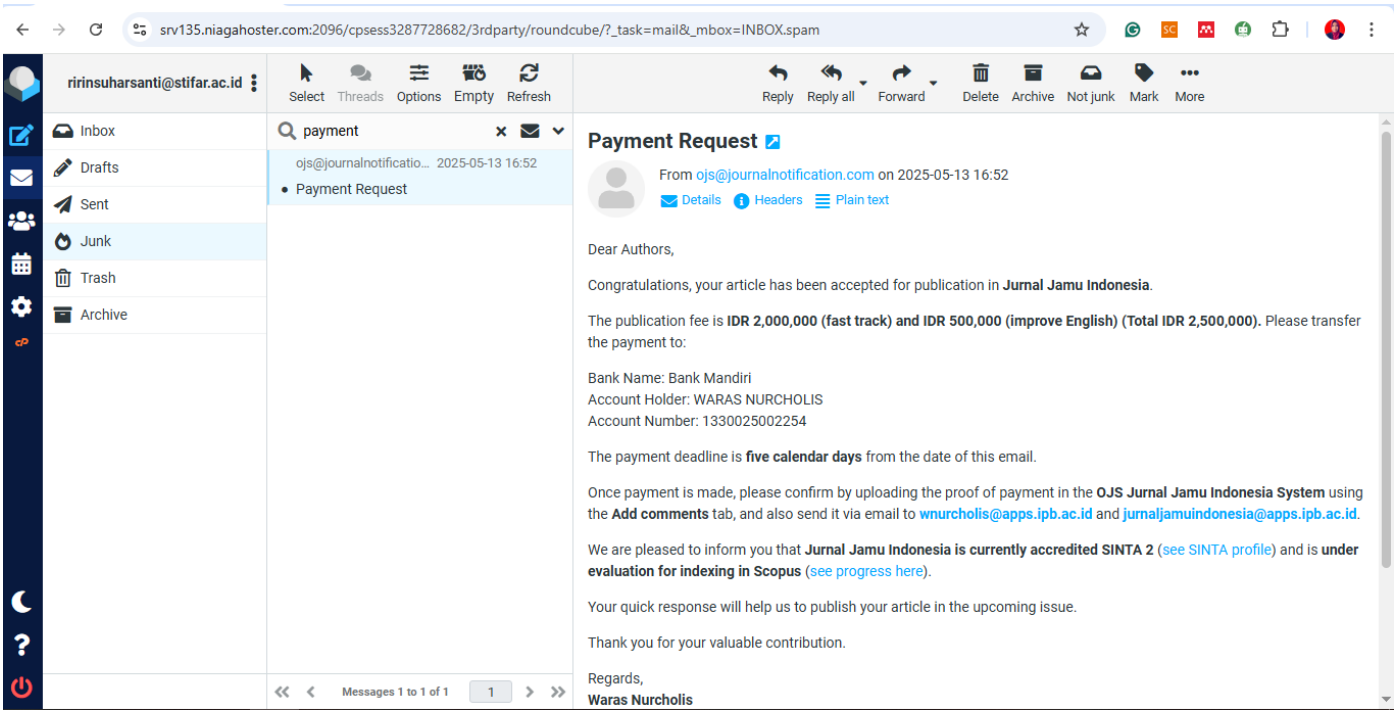

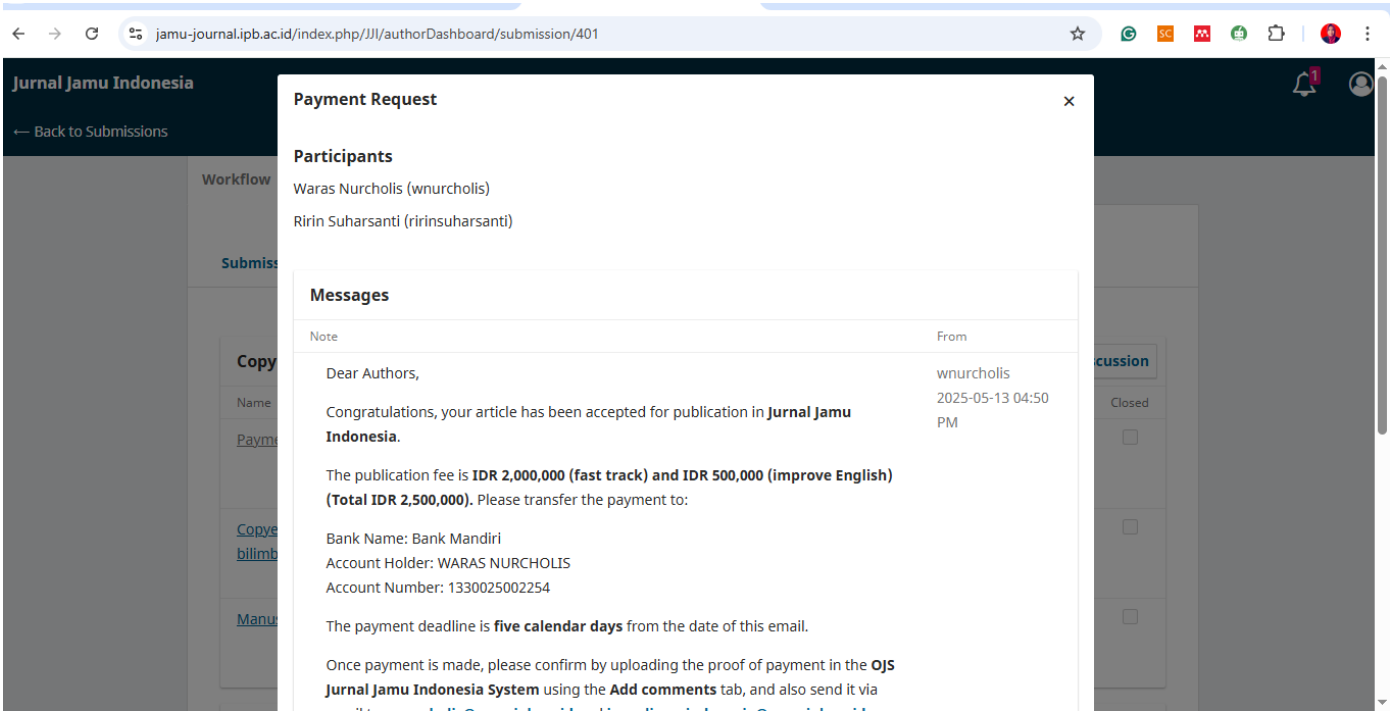

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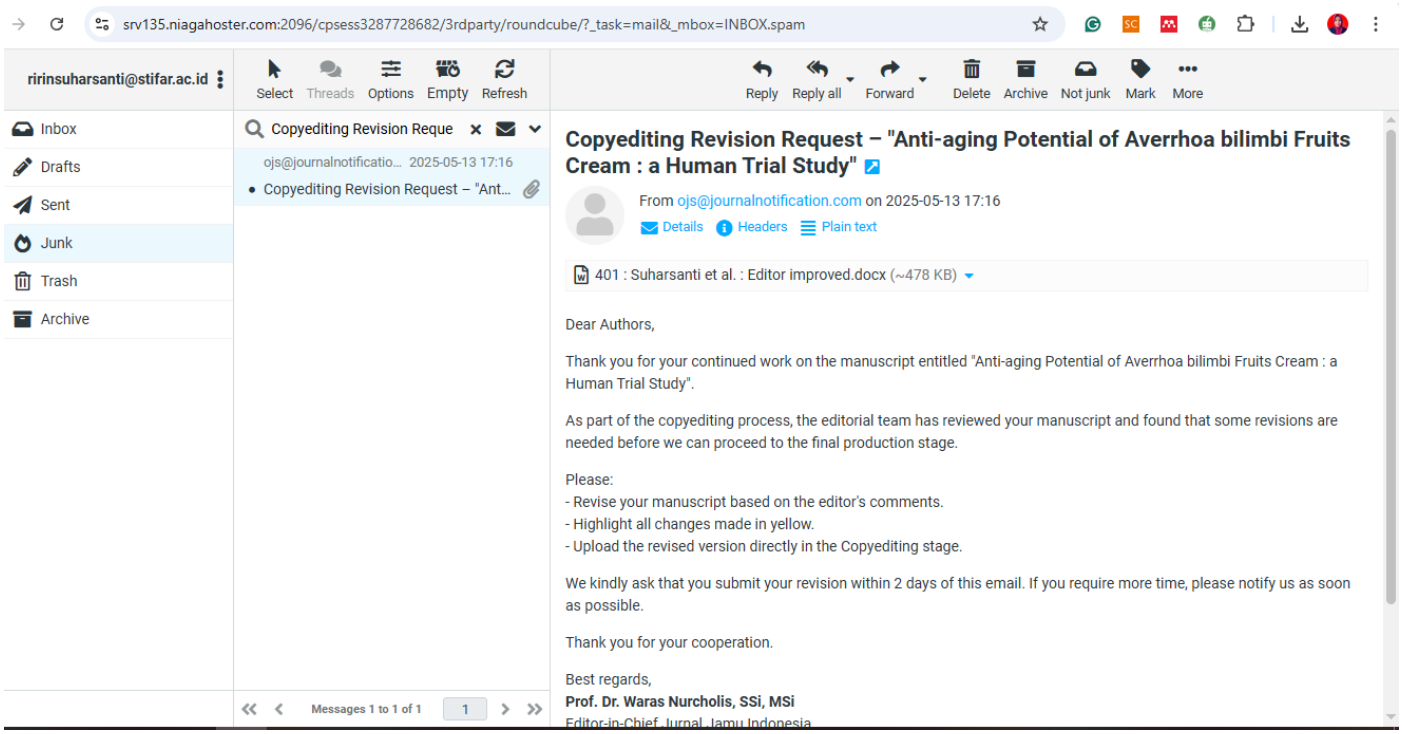

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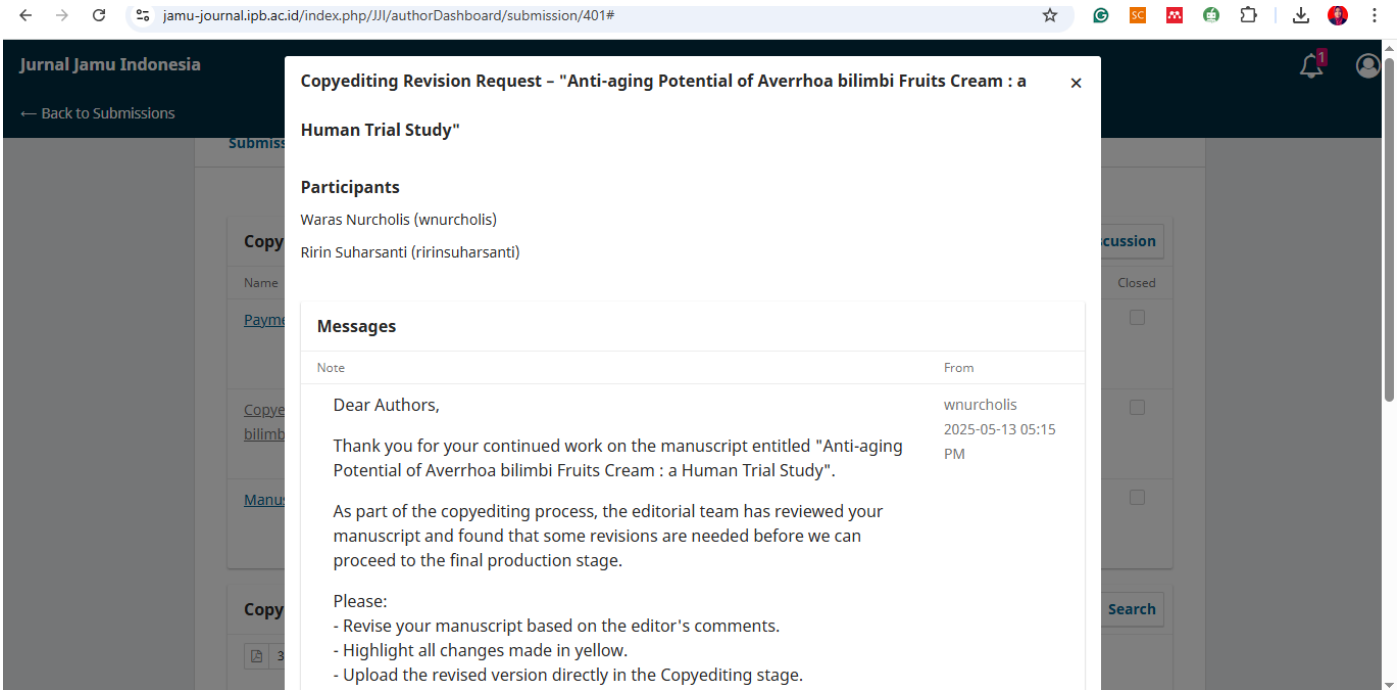

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Participants

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Ririn Suharsanti (ririnsuharsanti)

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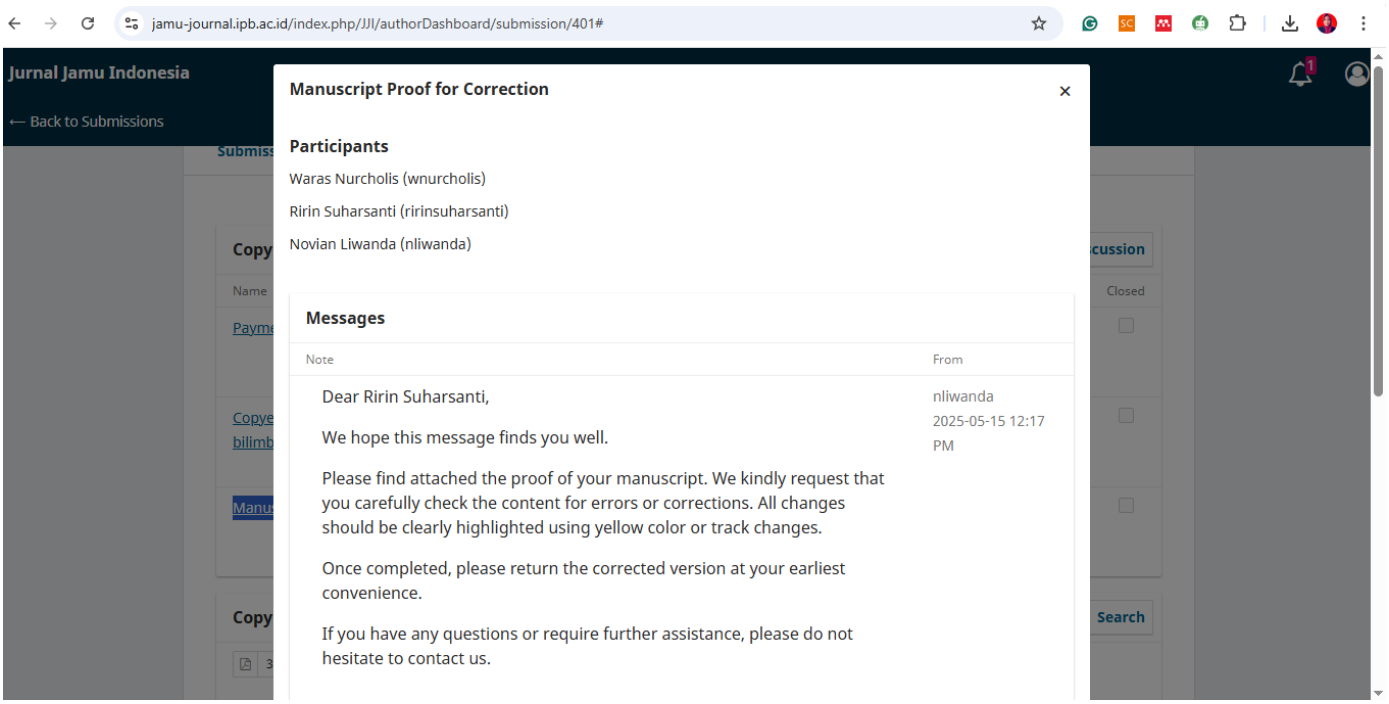

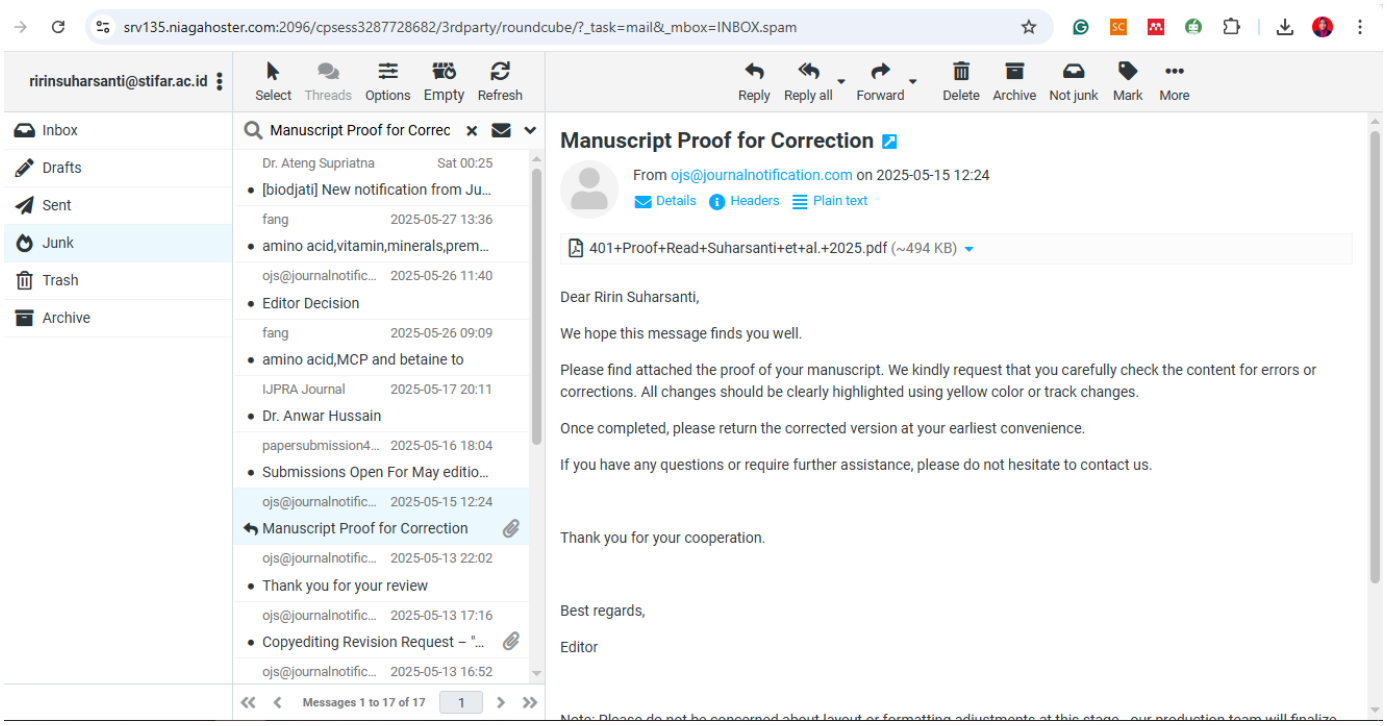

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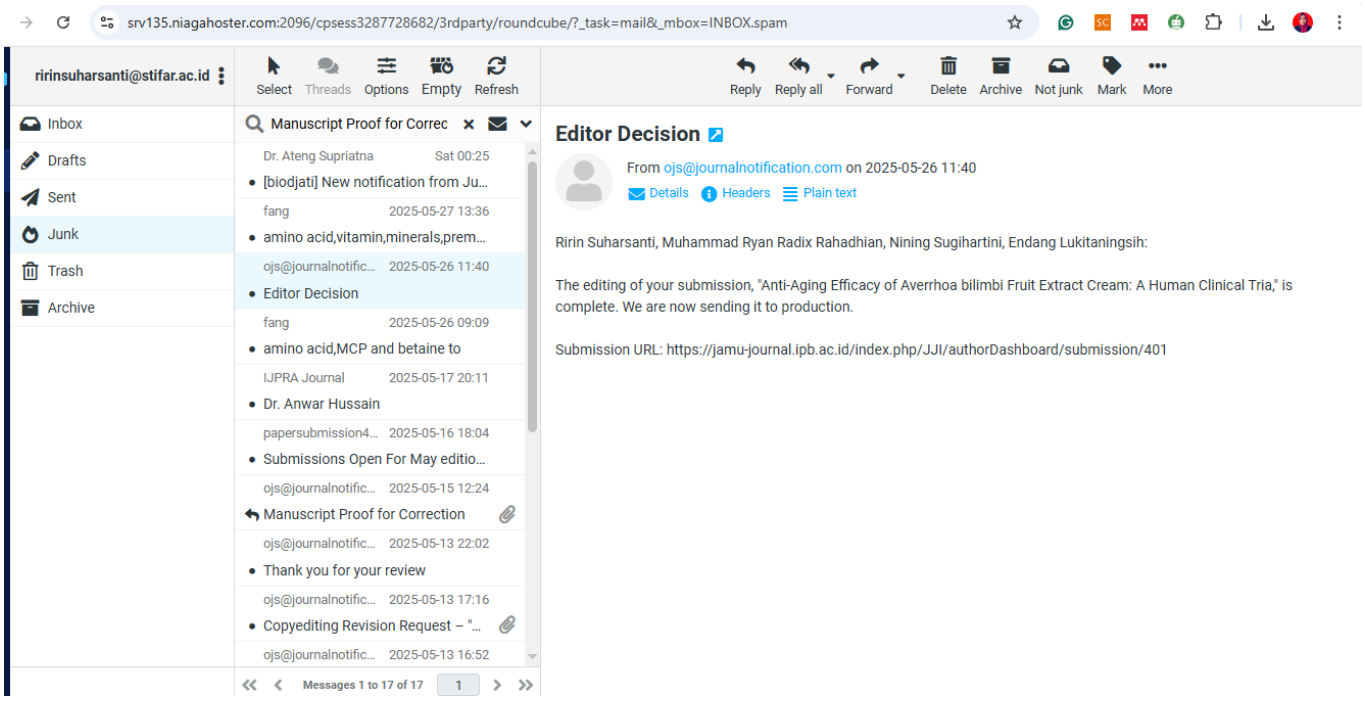

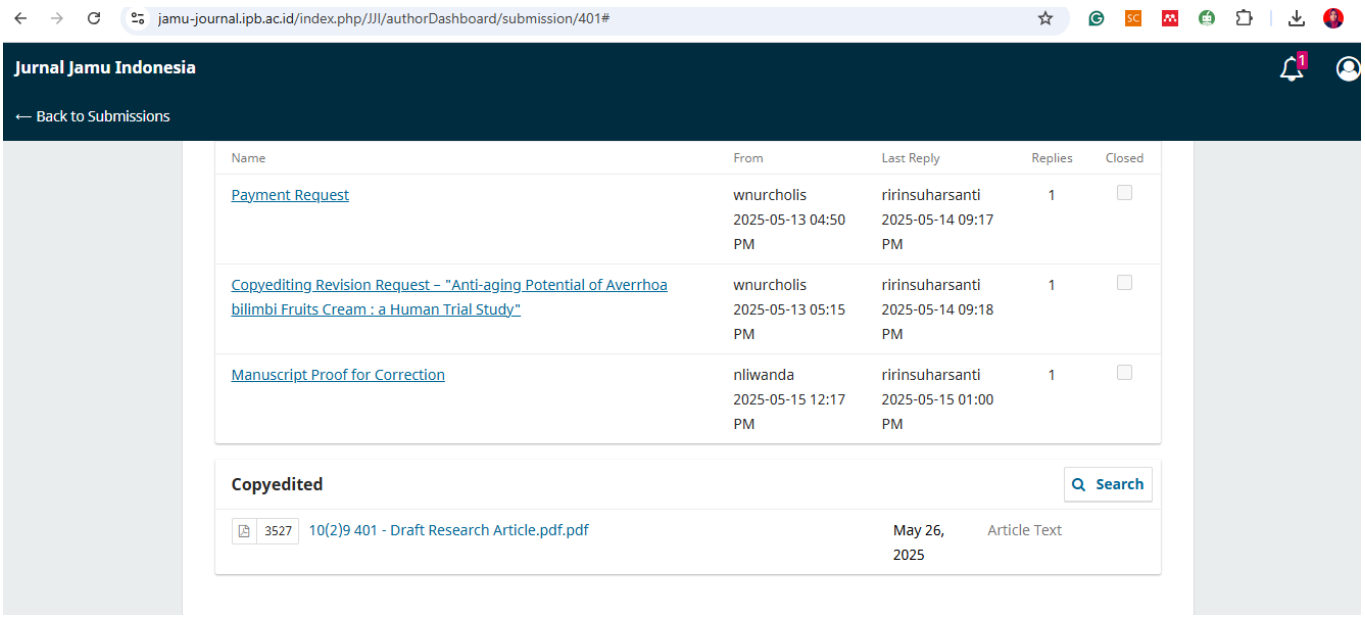

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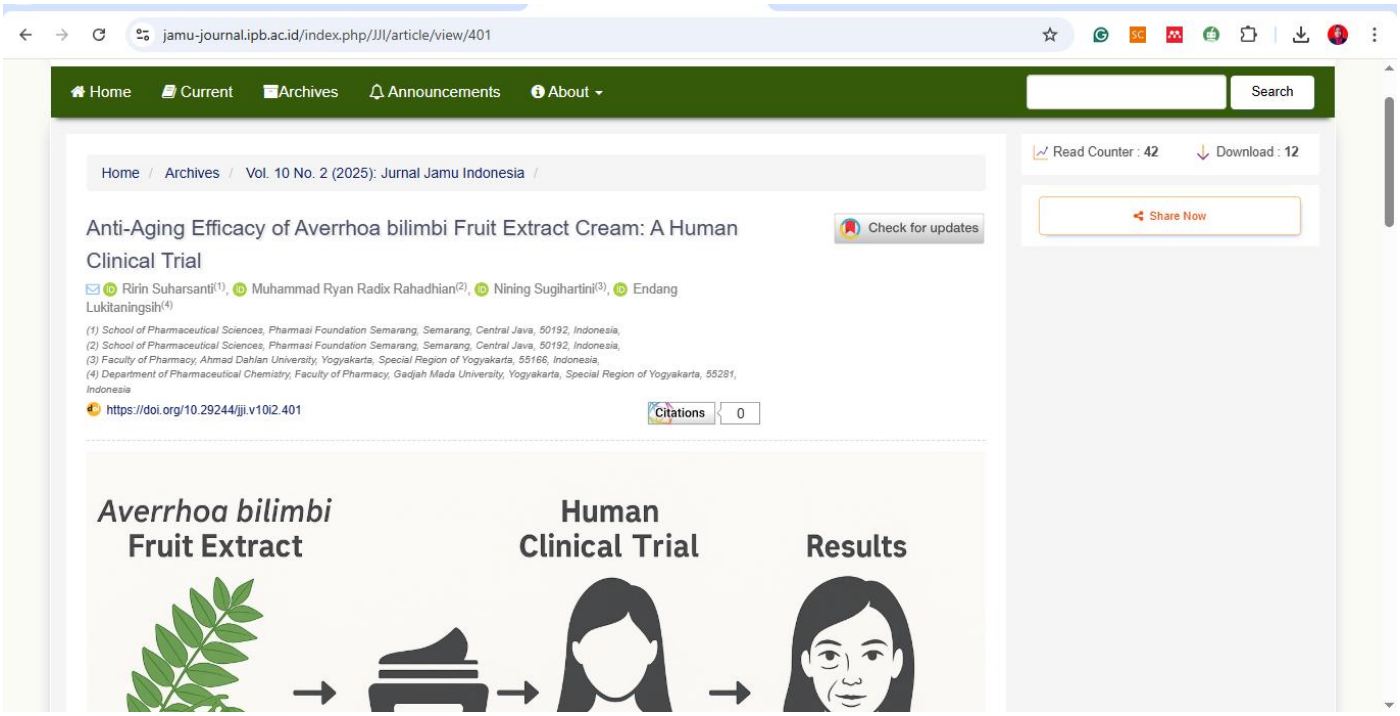

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Submission of Manuscript

Anti-aging Potential of *Averrhoa bilimbi* Fruits Cream : a Human Trial Study
Potensi Antipenuaan dari Krim Buah Belimbing Wuluh : Percobaan pada Manusia

Ririn Suharsanti[1]*, Muhammad Ryan Radix Rahadhian[1], Nining Sugihartini[2], Endang Lukitaningsih[3]
[1]STIFAR Yayasan Pharmasi Semarang, Semarang, Central Java, 50192, Indonesia
[2]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, 55166, Indonesia
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, 55281, Indonesia
Corresponding author: ririnsuharsanti@stifar.ac.id, 082136923586

Abstract

Ultraviolet (UV) light can be a major factor in skin problems such as aging, erythema, hyperpigmentation, wrinkles, dermatitis and skin cancer. Natural chemicals such as polyphenols as flavonoids from natural ingredients can be an alternative anti-aging products. Development of dosage forms in the form of anti-aging cream is needed so that it can be used by the public. The aim of this research is to determine the concentration of *Averrhoa bilimbi* extract that can provide good anti-aging properties. *Averrhoa bilimbi* was extracted with 70% ethanol by remaceration. W/O cream preparation was made with various concentrations of F1 (1%), F2 (3%), and F3 (5%) with *Averrhoa bilimbi* extracts. The cream was tested for characteristics such as organoleptic, homogeneity, pH and viscosity. Irritation test was conducted on animals. All creams were also evaluated for its anti-aging effects with parameters of sebum, moisture, pigment, pore, elasticity, and collagen fibers with skin analyzer on human subjects who had used the cream for 30 days. The results showed that F3 has the best anti-aging effect by increasing moisture, reducing pigmentation, shrinking pores, and increasing skin elasticity. After statistical testing of t-test on each anti-aging parameter, F3 anti-aging cream has been proven to be effective in having the potential to reduce skin pores and increase skin elasticity.

Keywords: *Averrhoa bilimbi*, Cream Formulation, Anti-aging, Human Trial

Abstrak

Sinar ultraviolet (UV) dapat menjadi faktor utama dalam masalah kulit seperti penuaan dini, eritema, hiperpigmentasi, keriput, dermatitis dan kanker kulit. Zat kimia alami seperti polifenol dan flavonoid dari bahan alam dapat menjadi alternatif produk anti-aging. Pengembangan bentuk sediaan berupa krim anti-aging diperlukan agar dapat digunakan oleh masyarakat. Tujuan dari penelitian ini adalah untuk menentukan konsentrasi ekstrak belimbing wuluh yang dapat memberikan sifat anti-aging yang baik. Belimbing wuluh diekstraksi dengan etanol 70% secara remaserasi. Sediaan krim A/M dibuat dengan berbagai konsentrasi F1 (1%), F2 (3%), dan F3 (5%) dengan ekstrak belimbing wuluh. Krim diuji karakteristiknya seperti organoleptik, homogenitas, pH dan

viskositas. Uji iritasi dilakukan pada hewan. Semua krim juga dievaluasi efek anti-penuaannya dengan parameter sebum, kelembaban, pigmen, pori-pori, elastisitas, dan serat kolagen dengan skin analyzer pada subjek manusia yang telah menggunakan krim selama 30 hari. Hasil penelitian menunjukkan bahwa F3 (5%) memiliki efek anti-penuaan terbaik dengan meningkatkan kelembaban, mengurangi pigmentasi, mengecilkan pori-pori, dan meningkatkan elastisitas kulit. Setelah pengujian statistik t test pada masing-masing parameter anti-aging, krim F3 telah terbukti efektif memiliki potensi untuk memperkecil pori-pori kulit dan meningkatkan elastisitas kulit.
Kata Kunci: *Averrhoa bilimbi*, Formulasi krim, Antipenuaan, Percobaan pada Manusia

Introduction

Skin aging is accelerated by 80% in areas exposed to sunlight (ultraviolet radiation), a process known as photoageing and occurs primarily on the face, neck, hands, and lower arms and legs (Aramo, 2012). Photoaging occurs due to a combination of short wavelengths (UVB) that hit the outer layer of the skin (epidermis) and long wavelengths (UVA) to the middle layer (dermis) (Binic et al., 2013). This exposure results in direct damage to the skin cell nucleus in the formation of reactive oxygen species (ROS) and other free radicals and reacts with important molecules in connective tissue and cell membranes. Clinically, acute UV-induced skin damage is seen as erythema, edema, and blistering of the sunburned skin followed by peeling, then tanning (a sign of long-term damage). Characteristics of skin aging for Asian skin types are a decrease in sebum levels, increased pigment and wrinkles in the skin, and loss of skin moisture (Dayan, 2008).

Cosmetic products have one of the benefits to protect the skin because of the ingredients they contain, so they can affect the biological function of the skin. Some plant extracts and antioxidants obtained from natural sources are able to prevent aging and can improve skin health. To prevent skin from aging or wrinkles, natural phytochemical sources are preferred. Plant extracts rich in secondary metabolites, such as flavonoids, phenolic acids, saponins and alkaloids, which have collagen synthesis activity, are being widely used for the development of topical anti-aging skin cosmetic products (Suharsanti et al., 2019). One of the natural sources that contains phenolic compounds, flavonoids is *Averrhoa bilimbi* fruit.

The total phenolic content of *Averrhoa bilimbi* fruits extract is 19.8022 ± 1.6696 mg GAE/g more than leaves ethanolic extract 6.5886±0.0590 mg GAE/g and total flavonoids 24.7458 ± 0.3291 mg RE/g more than leaves ethanolic extract 13.3126±0.5695 mg RE/g. The SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can be described in the category of sunblock assessment with maximum protection. *Averrhoa bilimbi* fruit extract has an IC_{50} value of antioxidant activity of 2.33 ± 0.33 mg/ml and tyrosinase inhibitor activity with an IC_{50} value of 186.85 ± 9.37 mg/mL (Suharsanti et al., 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa bilimbi* fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has an IC_{50} value of 74,625 ppm which is included in the category of strong antioxidants (Kusuma et al., 2023). In other research, The test results of antioxidant activity with the DPPH method using the most effective UV-Vis spectrophotometer as the antioxidant cream of the *Averrhoa bilimbi* leaves extract has a value of IC50 0.118 ppm and vitamin C as comparator has a value of IC_{50} 0.0327 ppm (Ariem et al., 2020). Shooting gel preparation with a combination of *Aloe vera* and *Averrhoa bilimbi* fruits formula I (85%) can increase the moisture value of human skin between before and after use (Ariani & Suharsanti, 2018a). Cream containing of 2% Bilimbi leaf extract (*Averrhoa bilimbi*) has favorable physical characteristics and effectively heals incision wounds in male white mice (Suena et al., 2024). The irritation test showed that all concentrations of the shooting gel preparation formula didn't cause irritation to the skin of mice (Ariani & Suharsanti, 2018b). Based on this, *Averrhoa bilimbi* fruit which has been proven to contain efficacious compounds and has activity as a skin whitener in vitro needs to be developed into an anti-aging skin cosmetic preparation.

Methods

a. Material

Averrhoa bilimbi dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin, stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV.

Setia Jaya Distributor, Indonesia), New Zealand Rabbits aged 12-16 weeks, Voluneteers include subjects aged 19–25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State), skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

b. Extraction of *Averrhoa bilimbi*

The 200 g dried and powdered fruits were remaserated with 70% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract (Rahardhian et al., 2019).

c. Formula Preparation

The extract obtained was made into cream preparations with concentrations of 1, 3, and 5%. The formula preparation of *Averrhoa bilimbi* extract can be seen in table 1.

Averrhoa bilimbi Fruits extract cream preparation is made by melting method. Water-soluble ingredients consisting of TEA, nipagin, glycerin are mixed and heated in distilled water at a temperature of 70°C. The oil phase consisting of stearic acid, cetyl alcohol, stearyl alcohol, and nipasol is heated at the same temperature. After that, the water phase is added little by little into the oil phase mixture while stirring until a homogeneous cream mass is formed. After that, the extract is added and mixed until homogeneous.

d. Physical Characteristics of Anti-aging Cream

Organoleptic Test

This examination is carried out by visually viewing the preparation including form, color and odor. This aims to determine the visual physical properties of cream (Ariem et al., 2020)

Homogeneity Test

This examination is carried out by placing the preparation between two glass objects and then observing it. The purpose of this test is to determine the mixing between each particle of the preparation made. The requirements for this test are that there are no visible coarse particles and they are perfectly homogeneously mixed (Ariem et al., 2020).

pH Test

Determination of the pH of the preparation is done using a digital pH meter solution. A good clay stick has a pH of 4.5-6.5 which is the ideal pH for topical preparations. This is in line with the pH of the skin which ranges from 4.5-6.5 (Ariem et al., 2020).

Viscosity Test

Determination of the viscosity of the cream preparation was carried out using a digital brookfield viscometer using the R7 spindle and by knowing the changes in viscosity in each cream formula. Reading the viscosity results in Cp (Ramdhan & Yusuf, 2023).

e. Ethical Clearance

Ethical clearance is a written ethical eligibility issued by the Medical/Health Research Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang with the number No. 313/V/2019/Bioethics Commission after meeting the established requirements. The creation of ethical clearance is necessary because this study uses living subjects, test animals and humans, so it is necessary to ensure that this study has met the principle of respecting human dignity.

f. Irritation Test on test animals

Irritation Test was conducted with the help of New Zealand Rabbits aged 12-16 weeks with the OECD method. The fur of the back and sides was shaved carefully ± 24 hours before application. The area is about 6 cm^2. Apply 0.5 g of cream to each formula. Wrap the area with a semi-occlusive bandage (half-closed, but still allows air exchange). Observe the animal after 1 hour, 24 hours, 48 hours, and 72 hours directly to the open skin. Irritation tests were also conducted on humans with the same treatment. Observe the presence of erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring System) according to table 2. Calculate the average score for each parameter at 24, 48, and 72 hours (OECD, 2021). Irritation conditions were also observed during application of the cream to humans during the initial 3 days of use.

g. Informed Consent

In this study, the subject was asked to sign an informed consent. The informed consent statement contains confirmation from the research subject that the person understands the research process, and rights including the right to refuse or withdraw from the study without any negative consequences, and an understanding that participation in the study is voluntary. Inclusion criteria include subjects aged 19–25 years, subjects not consuming skin vitamins or mineral supplements, all subjects in good condition based on health history. Exclusion criteria are subjects with degenerative diseases, skin diseases, inflammatory diseases, or consuming drugs, antioxidants, vitamins, or health supplements, subjects who are pregnant or breastfeeding. Signature of the research subject, date, place, and others if needed.

h. Human Trial of Anti-aging Cream

Anti-aging testing of *Averrhoa bilimbi* Fruits Cream preparation was conducted for 30 days with 24 volunteer. On the first day, the volunteer's skin was checked using a skin analyzer performed by a doctor at the Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia) to determine the skin condition before treatment, after 30 days it was checked again using a skin analyzer. For 30 days, the cream was used on the back of the volunteer's hand (according to the formula group) every morning and evening. Checks by the clinic's doctor included aging parameters such as sebum, moisture, pigment, pore, elasticity, and collagen fibers. To control the volunteer's compliance, every day the volunteer was required to check their skin with a portable skin analyzer that can check the water content and oil content of the volunteer.

i. Analysis Data

The results of each skin parameter before and after using the cream for 30 days were statistically tested using a paired t-test at each concentration. From the three formulas, one formula was chosen that had the best anti-aging effect.

Results and Discussion

a. Characteristics of Anti-aging Cream

Anti-aging cream preparations F1, F2, and F3 are semi-solid, have a fragrant odor, and have colors ranging from pale yellow to deep yellow. Anti-

aging cream preparations F1, F2, and F3 are homogeneous. The results of organoleptic and homogeneity tests can be seen in table 3.

The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range. The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus et al., 2024). So all cream preparation formulas have met the pH and viscosity quality requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

b. Irritation Test of Anti-aging Cream

The results of the irritation test on test animals using rabbits and human hand skin after 72 hours did not find any signs of irritation such as redness (erythema) and swelling (edema) (OECD, 2021). Skin reactions that have been assessed quantitatively using the Draize scale can be seen in the table 4. Based on table 4, it is proven that the *Averrhoa bilimbi* fruit cream doesn't cause irritation.

c. Human Trial of Anti-aging Cream

The study continued by observing the anti-aging parameters on the use of human hand skin. because no signs of irritation were found in the trial use of anti-aging cosmetics. After using the anti-aging cosmetics for 30 days, the results showed that the cosmetic preparation affected the condition of sebum, moisture, pigmentation, pore diameter, skin elasticity and collagen fibers. There was a decrease in pores and skin pigment after using the anti-aging cream for 30 days and is shown in Figure 2.

Skin Sebum

Sebum is part of the oil found on the surface of the skin. Sebum is produced by the sebaceous glands, or oil glands, and plays a role in helping to keep the skin and hair moist (Jadoon et al., 2015). Its components consist of

triglycerides, free fatty acids, wax esters, squalene, cholesterol esters, and cholesterol. Too much sebum can cause body odor. Sebum also has other roles for the skin, including maintaining moisture, skin flexibility, and also working as a skin protector from bacteria. However, high sebum content will clog pores and mix with various types of dirt. If left continuously, acne will eventually appear (Mahmood et al., 2010).

The skin sebum condition was reduced well after using F1 anti-aging cream compared to F2 and F3. The increase in sebum levels after the use of bases and formulations in F2 and F3 may be due to the oily nature of the W/O emulsion which has a thick oily liquid of stearic acid and glycerin (Rowe et al., 2006). Increased sebum also occurs in the topical cream of *Calendula officinalis* Extract formula due to the W/O emulsion type uses liquid paraffin (Akhtar et al., 2011). The results of the statistical test with paired t test on each concentration (base, F1, F2, F3) with baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin sebum condition (sig>0.05).

Skin Moisture

Moisture barrier is the outer layer of the skin that provides protection to maintain water and moisture in the skin, and protects the skin from external irritants such as dust and bacteria (Akhtar et al., 2011; Jadoon et al., 2015). The moisture barrier layer is made of lipids (oils) that bind skin cells, and when this layer is intact and undisturbed, this layer functions to make your skin feel soft and well-groomed. An example of an intact moisture barrier layer can be seen on baby skin that is supple, smooth, and soft. When the moisture barrier is disturbed or damaged, there will be small, invisible cracks in the skin. Although invisible, through these cracks, skin moisture can escape and disappear, and the danger of irritation can enter the skin more easily. As a result, your skin, which has lost its 'protection', will feel dry, 'tight', and become more vulnerable and sensitive (Sugihartini, 2017).

F3 anti-aging cream is able to increase skin moisture better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. F3 has

increased skin moisture by 22,34%. Although F3 has been able to increase skin moisture, statistics show something different. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on skin moisture because sig>0.05. Several studies on cream formulas from extracts have also been able to increase skin moisture, such as in anti-aging potential of a cream containing milk thistle extract. The increase in skin moisture after application of the formulation may be due to the flavonoids from milk thistle because flavonoids increase the water content in the skin due to swelling of corneocytes on the skin surface (Rasul & Akhtar, 2012). Due to the presence of vitamin E and essential fatty acids, *Sauropus Androgynus* leaf extract may help moisture retention in the epidermis and resistance to dryness (Siallagan et al., 2024). *Averrhoa bilimbi* fruit also contains phenolic and flavonoid content which may also have an effect on increasing skin moisture activity (Rahardhian et al., 2020). With the paired sample t-test it was clear that a significant difference in skin moisture values was produced at week 2 when the base was compared with the formulation (Akhtar et al., 2011).

Skin Pigment

Pigmentation or another name for hyperpigmentation is one of the skin problems where dark spots appear on some areas of the skin. These dark spots cause the overall skin color to become uneven. Skin pigmentation itself can be caused by several reasons. Among them are environmental pollution, UV rays, hormonal problems or other health problems. Hyperpigmentation is a skin condition caused by an increase in melanin, a substance in the body that is responsible for skin coloring (pigment)(Ohguchi et al., 2003). When a person is healthy, their skin color will appear normal. In cases of illness or injury, a person's skin can change color, becoming darker (hyperpigmentation) or lighter (hypopigmentation). Tyrosinase is the key responsible enzyme for synthesis of melanins (Khan et al., 2014; Nur et al., 2017). The reason is that *Averrhoa bilimbi* contains tyrosinase inhibitors, which have skin depigmenting effects (Rahardhian & Suharsanti, 2019).

F3 anti-aging cream is able to reduce skin pigmentation better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. Although F3 appears to be the most effective in reducing skin pigmentation, results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin pigmentation because sig>0.05. This may be due to the time the cream was applied was not long enough, in other studies the depigmentation effect appeared very well after 8 weeks (Khan et al., 2014).

Skin Pores

Basically, pores are the opening of follicles (gland pockets) to the surface of the skin. These pores function as an exit for oil and hair growth. In other words, pores are small openings in the skin that can pass good skin oil (also known as sebum) and hair to reach the surface of the skin. Pores are said to be large if their size is larger than the average hair follicle. Some of the impacts experienced when skin pores enlarge include excess oil. The smaller the pore value, the better the skin quality (Aramo, 2012). Conversely, if you have large pores, then the hair or fur that grows on the skin will increase. From the research data above, it is proven that using the cream for 1 month can shrink pores (Sugihartini, 2017).

F3 anti-aging cream is able to reduce the diameter of skin pores better than F1 and F2. Based on figure 2, F3 has the ability to shrink pores by up to 54.18%. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that anti-aging F2 and F3 cream has an effect on the condition of skin pores because sig <0.05. It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have anti-aging activity in shrinking pores.

Skin Elasticity

F3 anti-aging cream is able to improve skin elasticity better than F1 and F2. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that F3 anti-aging cream has an effect on skin elasticity because sig <0.05. One of the

characteristics of healthy, fresh and always young skin is having good elasticity. Another characteristic is that when touched, the facial skin feels fresh and supple. The use of F3 cream can increase skin elasticity by up to 16,85% compared to the first day. In other hand, The W/O cream of flower extract of *Calendula officinalis* exhibited aptitude of stimulating skin tightness and improved skin elasticity leading to delayed aging process (Jadoon et al., 2015).

In areas that are often exposed to direct sunlight, changes such as wrinkles and loss of skin elasticity will appear, causing the skin to sag and appear to be pulled downwards, causing the pores to widen (Sugihartini, 2017). Based on the statistical results of the pore data and skin elasticity, there is a match, where F3 has an effect on skin elasticity so that the skin pores also shrink.

Skin Collagen Fibers

Collagen is a type of fibrous and insoluble protein that is most abundant in the human body. Collagen is the main foundation of bones, skin, veins, and ligaments to give them structure and strength. Collagen is also found in many other parts of the body, including blood vessels, corneas and teeth (Jadoon et al., 2015). People who are deficient in collagen can be identified by the appearance of sagging skin, as well as the appearance of fine lines and wrinkles (Young, 2006).

F3 anti-aging cream is able to increase collagen fibers better than F1 and F2. The increase in skin collagen fibers due to F3 was only 1.12%. The results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on increasing skin collagen fibers because sig>0.05.

Conclusion

Based on the anti-aging parameter data that has been presented, anti-aging cream has the potential to reduce skin pores and increase skin elasticity and has been proven to be effective after statistical testing. While for other parameters, *Averrhoa bilimbi* cream with a concentration of 5% (F3) was reported to be the most able to improve skin conditions in a better direction but

has not been statistically effective. This can be caused by the application of anti-aging cream only 1 month of use and the skin conditions of each respondent are so diverse that of course this will affect these results.

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Table 1. *Averrhoa bilimb*i Fruits Cream Formula

Ingredients	F1(g)	F2(g)	F3 (g)
Extract	1	3	5
Stearic Acid	48	48	48
Cetyl Alcohol	6	6	6
Stearyl alcohol	4	4	4
Nipasol	1	1	1
Nipagin	1	1	1
Glycerin	54	54	54
TEA	4	4	4
Aquadest	Ad	Ad	Ad

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

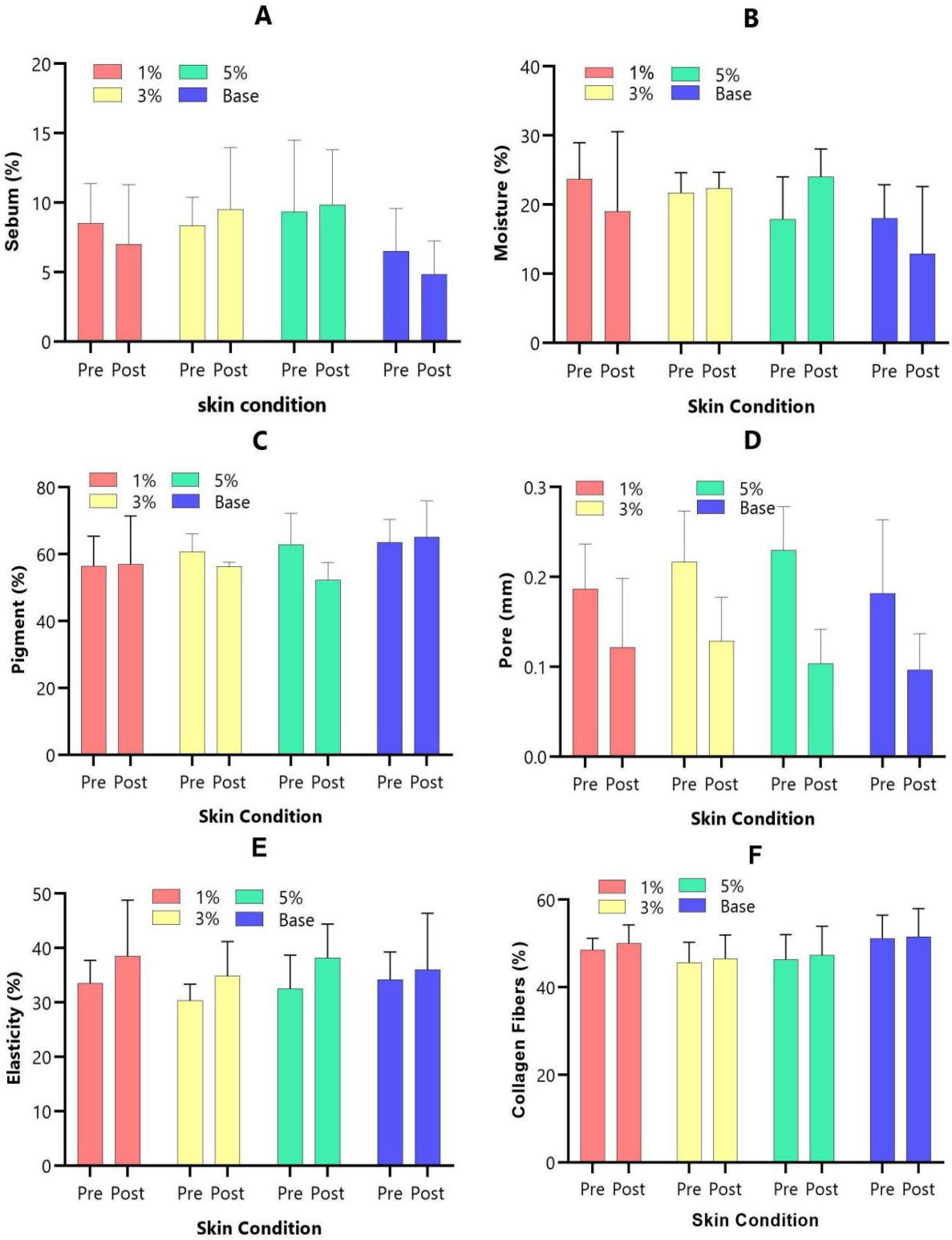
Table 3. Organoleptic and Homogeneity Test Results of Anti-aging Cream

Formula	Organoleptic			Homogenity
	Smell	**Form**	**Colour**	
Base	*Averrhoa bilimbi* fruit	Semi solid	White	Homogeneous
F1	*Averrhoa bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*Averrhoa bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*Averrhoa bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

Table 4. Results of Irritation Tests on Test Animals and Human

Formula	**Irritation Index**	
	Animal	**Human**
F1	0	0
F2	0	0
F3	0	0
Base	0	0

Figure 1. Results of pH (A) and Viscosity (B) tests on anti-aging cream


A
Sebum (%)
1%
3%
5%
Base
Pre Post
skin condition
B
Moisture (%)
Skin Condition
C
Pigment (%)
Skin Condition
D
Pore (mm)
Skin Condition
E
Elasticity (%)
Skin Condition
F
Collagen Fibers (%)
Skin Condition

546 **Figure 2.** Skin Condition Against Anti-aging Parameters after 30 days of cream
547 use **(A)** Sebum, **(B)** Moisture, **(C)** Pigment, **(D)** Pore, **(E)** Elasticity, **(F)**
548 Collagen Fibers
549

Manuscript revision request form reviewer 1

Anti-aging Potential of *Averrhoa bilimbi* Fruits Cream : a Human Trial Study
Potensi Antipenuaan dari Krim Buah Belimbing Wuluh : Percobaan pada Manusia

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Abstract

Ultraviolet (UV) light can be a major factor in skin problems such as aging, erythema, hyperpigmentation, wrinkles, dermatitis and skin cancer. Natural chemicals such as polyphenols as flavonoids from natural ingredients can be an alternative anti-aging products. Development of dosage forms in the form of anti-aging cream is needed so that it can be used by the public. The aim of this research is to determine the concentration of *Averrhoa bilimbi* extract that can provide good anti-aging properties. *Averrhoa bilimbi* was extracted with 70% ethanol by remaceration. W/O cream preparation was made with various concentrations of F1 (1%), F2 (3%), and F3 (5%) with *Averrhoa bilimbi* extracts. The cream was tested for characteristics such as organoleptic, homogeneity, pH and viscosity. Irritation test was conducted on animals. All creams were also evaluated for its anti-aging effects with parameters of sebum, moisture, pigment, pore, elasticity, and collagen fibers with skin analyzer on human subjects who had used the cream for 30 days. The results showed that F3 has the best anti-aging effect by increasing moisture, reducing pigmentation, shrinking pores, and increasing skin elasticity. After statistical testing of t-test on each anti-aging parameter, F3 anti-aging cream has been proven to be effective in having the potential to reduce skin pores and increase skin elasticity.
Keywords: *Averrhoa bilimbi*, Cream Formulation, Anti-aging, Human Trial

Abstrak

Sinar ultraviolet (UV) dapat menjadi faktor utama dalam masalah kulit seperti penuaan dini, eritema, hiperpigmentasi, keriput, dermatitis dan kanker kulit. Zat kimia alami seperti polifenol dan flavonoid dari bahan alam dapat menjadi alternatif produk anti-aging. Pengembangan bentuk sediaan berupa krim anti-aging diperlukan agar dapat digunakan oleh masyarakat. Tujuan dari penelitian ini adalah untuk menentukan konsentrasi ekstrak belimbing wuluh yang dapat memberikan sifat anti-aging yang baik. Belimbing wuluh diekstraksi dengan etanol 70% secara remaserasi. Sediaan krim A/M dibuat dengan berbagai konsentrasi F1 (1%), F2 (3%), dan F3 (5%) dengan ekstrak belimbing wuluh. Krim diuji karakteristiknya seperti organoleptik, homogenitas, pH dan viskositas. Uji iritasi dilakukan pada hewan. Semua krim juga dievaluasi efek anti-penuaannya dengan parameter sebum, kelembaban, pigmen, pori-pori, elastisitas, dan serat kolagen dengan skin analyzer pada subjek manusia yang telah menggunakan krim selama 30 hari. Hasil penelitian menunjukkan bahwa F3 (5%) memiliki efek anti-penuaan terbaik dengan meningkatkan kelembaban, mengurangi pigmentasi, mengecilkan pori-pori, dan meningkatkan elastisitas kulit. Setelah pengujian statistik t test pada masing-masing parameter anti-aging, krim F3 telah terbukti efektif memiliki potensi untuk memperkecil pori-pori kulit dan meningkatkan elastisitas kulit.

Kata Kunci: *Averrhoa bilimbi*, Formulasi krim, Antipenuaan, Percobaan pada Manusia

Introduction

Skin aging is accelerated by 80% in areas exposed to sunlight (ultraviolet radiation), a process known as photoageing and occurs primarily on the face, neck, hands, and lower arms and legs (Aramo, 2012). Photoaging occurs due to a combination of short wavelengths (UVB) that hit the outer layer of the skin (epidermis) and long wavelengths (UVA) to the middle layer (dermis) (Binic et al., 2013). This exposure results in direct damage to the skin cell nucleus in the formation of reactive oxygen species (ROS) and other free radicals and reacts with important molecules in connective tissue and cell membranes. Clinically, acute UV-induced skin damage is seen as erythema, edema, and blistering of the sunburned skin followed by peeling, then tanning (a sign of long-term damage). Characteristics of skin aging for Asian skin types are a decrease in sebum levels, increased pigment and wrinkles in the skin, and loss of skin moisture (Dayan, 2008).

Cosmetic products have one of the benefits to protect the skin because of the ingredients they contain, so they can affect the biological function of the skin. Some plant extracts and antioxidants obtained from natural sources are able to prevent aging and can improve skin health. To prevent skin from aging or wrinkles, natural phytochemical sources are preferred. Plant extracts rich in secondary metabolites, such as flavonoids, phenolic acids, saponins and alkaloids, which have collagen synthesis activity, are being widely used for the development of topical anti-aging skin cosmetic products (Suharsanti et al., 2019). One of the natural sources that contains phenolic compounds, flavonoids is *Averrhoa bilimbi* fruit.

The total phenolic content of *Averrhoa bilimbi* fruits extract is 19.8022 ± 1.6696 mg GAE/g more than leaves ethanolic extract 6.5886±0.0590 mg GAE/g and total flavonoids 24.7458 ± 0.3291 mg RE/g more than leaves ethanolic extract 13.3126±0.5695 mg RE/g. The SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can be described in the category of sunblock assessment with maximum protection. *Averrhoa bilimbi* fruit extract has an IC_{50} value of antioxidant activity of 2.33 ± 0.33 mg/ml and tyrosinase inhibitor activity with an IC_{50} value of 186.85 ± 9.37 mg/mL (Suharsanti et al., 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa bilimbi* fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has

an IC_{50} value of 74,625 ppm which is included in the category of strong antioxidants (Kusuma et al., 2023). In other research, The test results of antioxidant activity with the DPPH method using the most effective UV-Vis spectrophotometer as the antioxidant cream of the *Averrhoa bilimbi* leaves extract has a value of IC50 0.118 ppm and vitamin C as comparator has a value of IC_{50} 0.0327 ppm (Ariem et al., 2020). Shooting gel preparation with a combination of *Aloe vera* and *Averrhoa bilimbi* fruits formula I (85%) can increase the moisture value of human skin between before and after use (Ariani & Suharsanti, 2018a). Cream containing of 2% Bilimbi leaf extract (*Averrhoa bilimbi*) has favorable physical characteristics and effectively heals incision wounds in male white mice (Suena et al., 2024). The irritation test showed that all concentrations of the shooting gel preparation formula didn't cause irritation to the skin of mice (Ariani & Suharsanti, 2018b). Based on this, *Averrhoa bilimbi* fruit which has been proven to contain efficacious compounds and has activity as a skin whitener in vitro needs to be developed into an anti-aging skin cosmetic preparation.

Methods

a. Material

Averrhoa bilimbi dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin, stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor, Indonesia), New Zealand Rabbits aged 12-16 weeks, Voluneteers include subjects aged 19–25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State), skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

b. Extraction of *Averrhoa bilimbi*

The 200 g dried and powdered fruits were remaserated with 70% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract (Rahardhian et al., 2019).

c. Formula Preparation

The extract obtained was made into cream preparations with concentrations of 1, 3, and 5%. The formula preparation of *Averrhoa bilimbi* extract can be seen in table 1.

Averrhoa bilimbi Fruits extract cream preparation is made by melting method. Water-soluble ingredients consisting of TEA, nipagin, glycerin are mixed and heated in distilled water at a temperature of 70°C. The oil phase consisting of stearic acid, cetyl alcohol, stearyl alcohol, and nipasol is heated at the same temperature. After that, the water phase is added

little by little into the oil phase mixture while stirring until a homogeneous cream mass is formed. After that, the extract is added and mixed until homogeneous.

d. Physical Characteristics of Anti-aging Cream

Organoleptic Test

This examination is carried out by visually viewing the preparation including form, color and odor. This aims to determine the visual physical properties of cream (Ariem et al., 2020)

Homogeneity Test

This examination is carried out by placing the preparation between two glass objects and then observing it. The purpose of this test is to determine the mixing between each particle of the preparation made. The requirements for this test are that there are no visible coarse particles and they are perfectly homogeneously mixed (Ariem et al., 2020).

pH Test

Determination of the pH of the preparation is done using a digital pH meter solution. A good clay stick has a pH of 4.5-6.5 which is the ideal pH for topical preparations. This is in line with the pH of the skin which ranges from 4.5-6.5 (Ariem et al., 2020).

Viscosity Test

Determination of the viscosity of the cream preparation was carried out using a digital brookfield viscometer using the R7 spindle and by knowing the changes in viscosity in each cream formula. Reading the viscosity results in Cp (Ramdhan & Yusuf, 2023).

e. Ethical Clearance

Ethical clearance is a written ethical eligibility issued by the Medical/Health Research Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang with the number No. 313/V/2019/Bioethics Commission after meeting the established requirements. The creation of ethical clearance is necessary because this study uses living subjects, test animals and humans, so it is necessary to ensure that this study has met the principle of respecting human dignity.

f. Irritation Test on test animals

Comment[da1]: Are there any gender criteria in both animals and humans? If it is not needed, it can be explained in the method or discussion

Irritation Test was conducted with the help of New Zealand Rabbits aged 12-16 weeks with the OECD method. The fur of the back and sides was shaved carefully ± 24 hours before application. The area is about 6 cm^2. Apply 0.5 g of cream to each formula. Wrap the area with a semi-occlusive bandage (half-closed, but still allows air exchange). Observe the animal after 1 hour, 24 hours, 48 hours, and 72 hours directly to the open skin. Irritation

tests were also conducted on humans with the same treatment. Observe the presence of erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring System) according to table 2. Calculate the average score for each parameter at 24, 48, and 72 hours (OECD, 2021). Irritation conditions were also observed during application of the cream to humans during the initial 3 days of use.

g. Informed Consent

In this study, the subject was asked to sign an informed consent. The informed consent statement contains confirmation from the research subject that the person understands the research process, and rights including the right to refuse or withdraw from the study without any negative consequences, and an understanding that participation in the study is voluntary. Inclusion criteria include subjects aged 19–25 years, subjects not consuming skin vitamins or mineral supplements, all subjects in good condition based on health history. Exclusion criteria are subjects with degenerative diseases, skin diseases, inflammatory diseases, or consuming drugs, antioxidants, vitamins, or health supplements, subjects who are pregnant or breastfeeding. Signature of the research subject, date, place, and others if needed.

h. Human Trial of Anti-aging Cream

Anti-aging testing of *Averrhoa bilimbi* Fruits Cream preparation was conducted for 30 days with 24 volunteer. On the first day, the volunteer's skin was checked using a skin analyzer performed by a doctor at the Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia) to determine the skin condition before treatment, after 30 days it was checked again using a skin analyzer. For 30 days, the cream was used on the back of the volunteer's hand (according to the formula group) every morning and evening. Checks by the clinic's doctor included aging parameters such as sebum, moisture, pigment, pore, elasticity, and collagen fibers. To control the volunteer's compliance, every day the volunteer was required to check their skin with a portable skin analyzer that can check the water content and oil content of the volunteer.

i. Analysis Data

The results of each skin parameter before and after using the cream for 30 days were statistically tested using a paired t-test at each concentration. From the three formulas, one formula was chosen that had the best anti-aging effect.

Results and Discussion

Manuscript revision request form reviewer 1

a. Characteristics of Anti-aging Cream

Anti-aging cream preparations F1, F2, and F3 are semi-solid, have a fragrant odor, and have colors ranging from pale yellow to deep yellow. Anti-aging cream preparations F1, F2, and F3 are homogeneous. The results of organoleptic and homogeneity tests can be seen in table 3.

The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range. The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus et al., 2024). So all cream preparation formulas have met the pH and viscosity quality requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

b. Irritation Test of Anti-aging Cream

The results of the irritation test on test animals using rabbits and human hand skin after 72 hours did not find any signs of irritation such as redness (erythema) and swelling (edema) (OECD, 2021). Skin reactions that have been assessed quantitatively using the Draize scale can be seen in the table 4. Based on table 4, it is proven that the *Averrhoa bilimbi* fruit cream doesn't cause irritation.

c. Human Trial of Anti-aging Cream

The study continued by observing the anti-aging parameters on the use of human hand skin. because no signs of irritation were found in the trial use of anti-aging cosmetics. After using the anti-aging cosmetics for 30 days, the results showed that the cosmetic preparation affected the condition of sebum, moisture, pigmentation, pore diameter, skin elasticity and collagen fibers. There was a decrease in pores and skin pigment after using the anti-aging cream for 30 days and is shown in Figure 2.

Skin Sebum

Sebum is part of the oil found on the surface of the skin. Sebum is produced by the sebaceous glands, or oil glands, and plays a role in helping to keep the skin and hair moist (Jadoon et al., 2015). Its components consist of triglycerides, free fatty acids, wax esters, squalene, cholesterol esters, and cholesterol. Too much sebum can cause body odor. Sebum also has other roles for the skin, including maintaining moisture, skin flexibility, and also

Comment[da2]: 1. Please add to the discussion the relationship between the characterization of the preparation and the test results on animals and humans

working as a skin protector from bacteria. However, high sebum content will clog pores and mix with various types of dirt. If left continuously, acne will eventually appear (Mahmood et al., 2010).

The skin sebum condition was reduced well after using F1 anti-aging cream compared to F2 and F3. The increase in sebum levels after the use of bases and formulations in F2 and F3 may be due to the oily nature of the W/O emulsion which has a thick oily liquid of stearic acid and glycerin (Rowe et al., 2006). Increased sebum also occurs in the topical cream of *Calendula officinalis* Extract formula due to the W/O emulsion type uses liquid paraffin (Akhtar et al., 2011). The results of the statistical test with paired t test on each concentration (base, F1, F2, F3) with baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin sebum condition (sig>0.05).

Skin Moisture

Moisture barrier is the outer layer of the skin that provides protection to maintain water and moisture in the skin, and protects the skin from external irritants such as dust and bacteria (Akhtar et al., 2011; Jadoon et al., 2015). The moisture barrier layer is made of lipids (oils) that bind skin cells, and when this layer is intact and undisturbed, this layer functions to make your skin feel soft and well-groomed. An example of an intact moisture barrier layer can be seen on baby skin that is supple, smooth, and soft. When the moisture barrier is disturbed or damaged, there will be small, invisible cracks in the skin. Although invisible, through these cracks, skin moisture can escape and disappear, and the danger of irritation can enter the skin more easily. As a result, your skin, which has lost its 'protection', will feel dry, 'tight', and become more vulnerable and sensitive (Sugihartini, 2017).

F3 anti-aging cream is able to increase skin moisture better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. F3 has increased skin moisture by 22,34%. Although F3 has been able to increase skin moisture, statistics show something different. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on skin moisture because sig>0.05. Several studies on cream formulas from extracts have also been able to increase skin moisture, such as in anti-aging potential of a cream containing milk thistle extract. The increase in skin moisture after application of the formulation may be due to the flavonoids from milk thistle because

flavonoids increase the water content in the skin due to swelling of corneocytes on the skin surface (Rasul & Akhtar, 2012). Due to the presence of vitamin E and essential fatty acids, *Sauropus Androgynus* leaf extract may help moisture retention in the epidermis and resistance to dryness (Siallagan et al., 2024). *Averrhoa bilimbi* fruit also contains phenolic and flavonoid content which may also have an effect on increasing skin moisture activity (Rahardhian et al., 2020). With the paired sample t-test it was clear that a significant difference in skin moisture values was produced at week 2 when the base was compared with the formulation (Akhtar et al., 2011).

Skin Pigment

Pigmentation or another name for hyperpigmentation is one of the skin problems where dark spots appear on some areas of the skin. These dark spots cause the overall skin color to become uneven. Skin pigmentation itself can be caused by several reasons. Among them are environmental pollution, UV rays, hormonal problems or other health problems. Hyperpigmentation is a skin condition caused by an increase in melanin, a substance in the body that is responsible for skin coloring (pigment)(Ohguchi et al., 2003). When a person is healthy, their skin color will appear normal. In cases of illness or injury, a person's skin can change color, becoming darker (hyperpigmentation) or lighter (hypopigmentation). Tyrosinase is the key responsible enzyme for synthesis of melanins (Khan et al., 2014; Nur et al., 2017). The reason is that *Averrhoa bilimbi* contains tyrosinase inhibitors, which have skin depigmenting effects (Rahardhian & Suharsanti, 2019).

F3 anti-aging cream is able to reduce skin pigmentation better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. Although F3 appears to be the most effective in reducing skin pigmentation, results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) didn’t show any effect of using anti-aging cream on skin pigmentation because sig>0.05. This may be due to the time the cream was applied was not long enough, in other studies the depigmentation effect appeared very well after 8 weeks (Khan et al., 2014).

Skin Pores

Basically, pores are the opening of follicles (gland pockets) to the surface of the skin. These pores function as an exit for oil and hair growth. In other words, pores are small openings in the skin that can pass good skin oil (also known as sebum) and hair to reach the surface of the skin. Pores are said to be large if their size is larger than the average hair

follicle. Some of the impacts experienced when skin pores enlarge include excess oil. The smaller the pore value, the better the skin quality (Aramo, 2012). Conversely, if you have large pores, then the hair or fur that grows on the skin will increase. From the research data above, it is proven that using the cream for 1 month can shrink pores (Sugihartini, 2017).

F3 anti-aging cream is able to reduce the diameter of skin pores better than F1 and F2. Based on figure 2, F3 has the ability to shrink pores by up to 54.18%. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that anti-aging F2 and F3 cream has an effect on the condition of skin pores because sig <0.05. It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have anti-aging activity in shrinking pores.

Skin Elasticity

F3 anti-aging cream is able to improve skin elasticity better than F1 and F2. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that F3 anti-aging cream has an effect on skin elasticity because sig <0.05. One of the characteristics of healthy, fresh and always young skin is having good elasticity. Another characteristic is that when touched, the facial skin feels fresh and supple. The use of F3 cream can increase skin elasticity by up to 16,85% compared to the first day. In other hand, The W/O cream of flower extract of *Calendula officinalis* exhibited aptitude of stimulating skin tightness and improved skin elasticity leading to delayed aging process (Jadoon et al., 2015).

In areas that are often exposed to direct sunlight, changes such as wrinkles and loss of skin elasticity will appear, causing the skin to sag and appear to be pulled downwards, causing the pores to widen (Sugihartini, 2017). Based on the statistical results of the pore data and skin elasticity, there is a match, where F3 has an effect on skin elasticity so that the skin pores also shrink.

Skin Collagen Fibers

Collagen is a type of fibrous and insoluble protein that is most abundant in the human body. Collagen is the main foundation of bones, skin, veins, and ligaments to give them structure and strength. Collagen is also found in many other parts of the body, including blood vessels, corneas and teeth (Jadoon et al., 2015). People who are deficient in collagen can be identified by the appearance of sagging skin, as well as the appearance of fine lines and wrinkles (Young, 2006).

F3 anti-aging cream is able to increase collagen fibers better than F1 and F2. The increase in skin collagen fibers due to F3 was only 1.12%. The results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on increasing skin collagen fibers because sig>0.05.

Conclusion

Based on the anti-aging parameter data that has been presented, anti-aging cream has the potential to reduce skin pores and increase skin elasticity and has been proven to be effective after statistical testing. While for other parameters, *Averrhoa bilimbi* cream with a concentration of 5% (F3) was reported to be the most able to improve skin conditions in a better direction but has not been statistically effective. This can be caused by the application of anti-aging cream only 1 month of use and the skin conditions of each respondent are so diverse that of course this will affect these results.

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Table 1. *Averrhoa bilimb*i Fruits Cream Formula

Ingredients	F1(g)	F2(g)	F3 (g)
Extract	1	3	5
Stearic Acid	48	48	48
Cetyl Alcohol	6	6	6
Stearyl alcohol	4	4	4
Nipasol	1	1	1
Nipagin	1	1	1
Glycerin	54	54	54
TEA	4	4	4
Aquadest	Ad	Ad	Ad

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

Table 3. Organoleptic and Homogeneity Test Results of Anti-aging Cream

Formula	Organoleptic			Homogenity
	Smell	**Form**	**Colour**	
Base	*Averrhoa bilimbi* fruit	Semi solid	White	Homogeneous
F1	*Averrhoa bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*Averrhoa bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*Averrhoa bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

Table 4. Results of Irritation Tests on Test Animals and Human

Formula	**Irritation Index**	
	Animal	**Human**
F1	0	0
F2	0	0
F3	0	0
Base	0	0

Manuscript revision request form reviewer 1

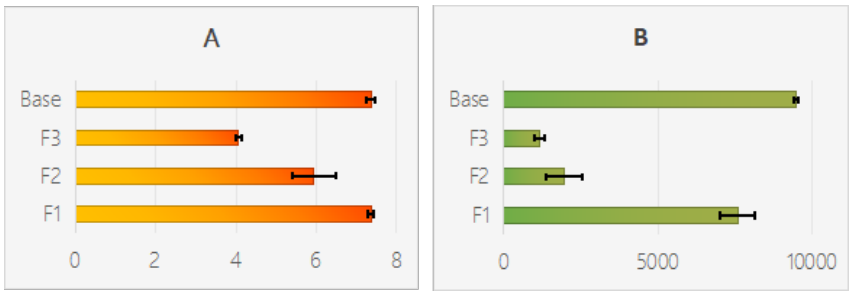

Figure 1. Results of pH (A) and Viscosity (B) tests on anti-aging cream

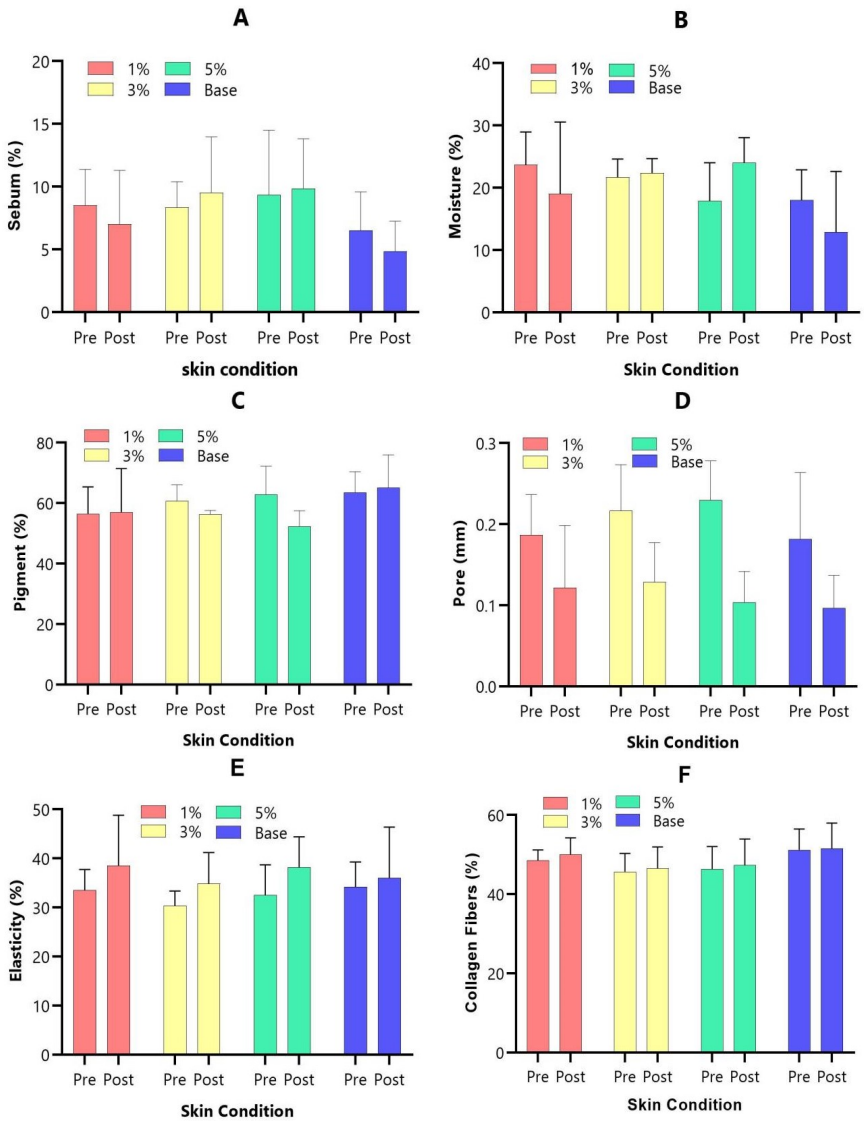

Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use **(A)** Sebum, **(B)** Moisture, **(C)** Pigment, **(D)** Pore, **(E)** Elasticity, **(F)** Collagen Fibers

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Anti-aging Potential of *Averrhoa bilimbi* Fruits Cream : a Human Trial Study

Potensi Antipenuaan dari Krim Buah Belimbing Wuluh : Percobaan pada Manusia

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Abstract

Ultraviolet (UV) light can be a major factor in skin problems such as aging, erythema, hyperpigmentation, wrinkles, dermatitis and skin cancer. Natural chemicals such as polyphenols as flavonoids from natural ingredients can be an alternative anti-aging products. Development of dosage forms in the form of anti-aging cream is needed so that it can be used by the public. The aim of this research is to determine the concentration of *Averrhoa bilimbi* extract that can provide good anti-aging properties. *Averrhoa bilimbi* was extracted with 70% ethanol by remaceration. W/O cream preparation was made with various concentrations of F1 (1%), F2 (3%), and F3 (5%) with *Averrhoa bilimbi* extracts. The cream was tested for characteristics such as organoleptic, homogeneity, pH and viscosity. Irritation test was conducted on animals. All creams were also evaluated for its anti-aging effects with parameters of sebum, moisture, pigment, pore, elasticity, and collagen fibers with skin analyzer on human subjects who had used the cream for 30 days. The results showed that F3 has the best anti-aging effect by increasing moisture, reducing pigmentation, shrinking pores, and increasing skin elasticity. After statistical testing of t-test on each anti-aging parameter, F3 anti-aging cream has been proven to be effective in having the potential to reduce skin pores and increase skin elasticity.

Keywords: *Averrhoa bilimbi*, Cream Formulation, Anti-aging, Human Trial

Abstrak

Sinar ultraviolet (UV) dapat menjadi faktor utama dalam masalah kulit seperti penuaan dini, eritema, hiperpigmentasi, keriput, dermatitis dan kanker kulit. Zat kimia alami seperti polifenol dan flavonoid dari bahan alam dapat menjadi alternatif produk anti-aging. Pengembangan bentuk sediaan berupa krim anti-aging diperlukan agar dapat digunakan oleh masyarakat. Tujuan dari penelitian ini adalah untuk menentukan konsentrasi ekstrak belimbing wuluh yang dapat memberikan sifat anti-aging yang baik. Belimbing wuluh diekstraksi dengan etanol 70% secara remaserasi. Sediaan krim A/M dibuat dengan berbagai konsentrasi F1 (1%), F2 (3%), dan F3 (5%) dengan ekstrak belimbing wuluh. Krim diuji karakteristiknya seperti organoleptik, homogenitas, pH dan viskositas. Uji iritasi dilakukan pada hewan. Semua krim juga dievaluasi efek anti-penuaannya dengan parameter sebum, kelembaban, pigmen, pori-pori, elastisitas, dan serat kolagen dengan skin analyzer pada subjek manusia yang telah menggunakan krim selama 30 hari. Hasil penelitian menunjukkan bahwa F3 (5%) memiliki efek anti-penuaan terbaik dengan meningkatkan kelembaban, mengurangi pigmentasi, mengecilkan pori-pori, dan meningkatkan elastisitas kulit. Setelah pengujian statistik t test pada masing-masing parameter anti-aging, krim F3 telah terbukti efektif memiliki potensi untuk memperkecil pori-pori kulit dan meningkatkan elastisitas kulit.

Kata Kunci: *Averrhoa bilimbi*, Formulasi krim, Antipenuaan, Percobaan pada Manusia

Introduction

Skin aging is accelerated by 80% in areas exposed to sunlight (ultraviolet radiation), a process known as photoageing and occurs primarily on the face, neck, hands, and lower arms and legs (Aramo, 2012). Photoaging occurs due to a combination of short wavelengths (UVB) that hit the outer layer of the skin (epidermis) and long wavelengths (UVA) to the middle layer (dermis) (Binic et al., 2013). This exposure results in direct damage to the skin cell nucleus in the formation of reactive oxygen species (ROS) and other free radicals and reacts with important molecules in connective tissue and cell membranes. Clinically, acute UV-induced skin damage is seen as erythema, edema, and blistering of the sunburned skin followed by peeling, then tanning (a sign of long-term damage). Characteristics of skin aging for Asian skin types are a decrease in sebum levels, increased pigment and wrinkles in the skin, and loss of skin moisture (Dayan, 2008).

Comment[L1]: What is the correct writing? photoaging

Comment[L2]: The same as above

Cosmetic products have one of the benefits to protect the skin because of the ingredients they contain, so they can affect the biological function of the skin. Some plant extracts and antioxidants obtained from natural sources are able to prevent aging and can improve skin health. To prevent skin from aging or wrinkles, natural phytochemical sources are preferred. Plant extracts rich in secondary metabolites, such as flavonoids, phenolic acids, saponins and alkaloids, which have collagen synthesis activity, are being widely used for the development of topical anti-aging skin cosmetic products (Suharsanti et al., 2019). One of the natural sources that contains phenolic compounds, flavonoids is *Averrhoa bilimbi* fruit.

The total phenolic content of *Averrhoa bilimbi* fruits extract is 19.8022 ± 1.6696 mg GAE/g more than leaves ethanolic extract 6.5886±0.0590 mg GAE/g and total flavonoids 24.7458 ± 0.3291 mg RE/g more than leaves ethanolic extract 13.3126±0.5695 mg RE/g. The SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can be described in the category of sunblock assessment with maximum protection. *Averrhoa bilimbi* fruit extract has an IC_{50} value of antioxidant activity of 2.33 ± 0.33 mg/ml and tyrosinase inhibitor activity with an IC_{50} value of 186.85 ± 9.37 mg/mL (Suharsanti et al., 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa bilimbi* fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has

an IC_{50} value of 74,625 ppm which is included in the category of strong antioxidants (Kusuma et al., 2023). In other research, The test results of antioxidant activity with the DPPH method using the most effective UV-Vis spectrophotometer as the antioxidant cream of the *Averrhoa bilimbi* leaves extract has a value of IC50 0.118 ppm and vitamin C as comparator has a value of IC_{50} 0.0327 ppm (Ariem et al., 2020). Shooting gel preparation with a combination of *Aloe vera* and *Averrhoa bilimbi* fruits formula I (85%) can increase the moisture value of human skin between before and after use (Ariani & Suharsanti, 2018a). Cream containing of 2% Bilimbi leaf extract (*Averrhoa bilimbi*) has favorable physical characteristics and effectively heals incision wounds in male white mice (Suena et al., 2024). The irritation test showed that all concentrations of the shooting gel preparation formula didn't cause irritation to the skin of mice (Ariani & Suharsanti, 2018b). Based on this, *Averrhoa bilimbi* fruit which has been proven to contain efficacious compounds and has activity as a skin whitener in vitro needs to be developed into an anti-aging skin cosmetic preparation.

Methods

a. Material

Averrhoa bilimbi dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin, stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor, Indonesia), New Zealand Rabbits aged 12-16 weeks, Voluneteers include subjects aged 19–25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State), skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

b. Extraction of *Averrhoa bilimbi*

The 200 g dried and powdered fruits were remaserated with 70% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract (Rahardhian et al., 2019).

Comment[L3]: Samples used by dried fruit or powder?

c. Formula Preparation

The extract obtained was made into cream preparations with concentrations of 1, 3, and 5%. The formula preparation of *Averrhoa bilimbi* extract can be seen in table 1.

Comment[L4]: citation

Averrhoa bilimbi Fruits extract cream preparation is made by melting method. Water-soluble ingredients consisting of TEA, nipagin, glycerin are mixed and heated in distilled water at a temperature of 70°C. The oil phase consisting of stearic acid, cetyl alcohol, stearyl alcohol, and nipasol is heated at the same temperature. After that, the water phase is added

little by little into the oil phase mixture while stirring until a homogeneous cream mass is formed. After that, the extract is added and mixed until homogeneous.

d. Physical Characteristics of Anti-aging Cream

Organoleptic Test

This examination is carried out by visually viewing the preparation including form, color and odor. This aims to determine the visual physical properties of cream (Ariem et al., 2020)

Homogeneity Test

This examination is carried out by placing the preparation between two glass objects and then observing it. The purpose of this test is to determine the mixing between each particle of the preparation made. The requirements for this test are that there are no visible coarse particles and they are perfectly homogeneously mixed (Ariem et al., 2020).

pH Test

Determination of the pH of the preparation is done using a digital pH meter solution. A good clay stick has a pH of 4.5-6.5 which is the ideal pH for topical preparations. This is in line with the pH of the skin which ranges from 4.5-6.5 (Ariem et al., 2020).

Viscosity Test

Determination of the viscosity of the cream preparation was carried out using a digital brookfield viscometer using the R7 spindle and by knowing the changes in viscosity in each cream formula. Reading the viscosity results in Cp (Ramdhan & Yusuf, 2023).

e. Ethical Clearance

Ethical clearance is a written ethical eligibility issued by the Medical/Health Research Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang with the number No. 313/V/2019/Bioethics Commission after meeting the established requirements. The creation of ethical clearance is necessary because this study uses living subjects, test animals and humans, so it is necessary to ensure that this study has met the principle of respecting human dignity.

f. Irritation Test on test animals

Irritation Test was conducted with the help of New Zealand Rabbits aged 12-16 weeks with the OECD method. The fur of the back and sides was shaved carefully ± 24 hours before application. The area is about 6 cm^2. Apply 0.5 g of cream to each formula. Wrap the area with a semi-occlusive bandage (half-closed, but still allows air exchange). Observe the animal after 1 hour, 24 hours, 48 hours, and 72 hours directly to the open skin. Irritation

tests were also conducted on humans with the same treatment. Observe the presence of erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring System) according to table 2. Calculate the average score for each parameter at 24, 48, and 72 hours (OECD, 2021). Irritation conditions were also observed during application of the cream to humans during the initial 3 days of use.

g. Informed Consent

In this study, the subject was asked to sign an informed consent. The informed consent statement contains confirmation from the research subject that the person understands the research process, and rights including the right to refuse or withdraw from the study without any negative consequences, and an understanding that participation in the study is voluntary. Inclusion criteria include subjects aged 19–25 years, subjects not consuming skin vitamins or mineral supplements, all subjects in good condition based on health history. Exclusion criteria are subjects with degenerative diseases, skin diseases, inflammatory diseases, or consuming drugs, antioxidants, vitamins, or health supplements, subjects who are pregnant or breastfeeding. Signature of the research subject, date, place, and others if needed.

h. Human Trial of Anti-aging Cream

Anti-aging testing of *Averrhoa bilimbi* Fruits Cream preparation was conducted for 30 days with 24 volunteer. On the first day, the volunteer's skin was checked using a skin analyzer performed by a doctor at the Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia) to determine the skin condition before treatment, after 30 days it was checked again using a skin analyzer. For 30 days, the cream was used on the back of the volunteer's hand (according to the formula group) every morning and evening. Checks by the clinic's doctor included aging parameters such as sebum, moisture, pigment, pore, elasticity, and collagen fibers. To control the volunteer's compliance, every day the volunteer was required to check their skin with a portable skin analyzer that can check the water content and oil content of the volunteer.

i. Analysis Data

The results of each skin parameter before and after using the cream for 30 days were statistically tested using a paired t-test at each concentration. From the three formulas, one formula was chosen that had the best anti-aging effect.

Results and Discussion

a. Characteristics of Anti-aging Cream

Anti-aging cream preparations F1, F2, and F3 are semi-solid, have a fragrant odor, and have colors ranging from pale yellow to deep yellow. Anti-aging cream preparations F1, F2, and F3 are homogeneous. The results of organoleptic and homogeneity tests can be seen in table 3.

The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range. The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus et al., 2024). So all cream preparation formulas have met the pH and viscosity quality requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

b. Irritation Test of Anti-aging Cream

The results of the irritation test on test animals using rabbits and human hand skin after 72 hours did not find any signs of irritation such as redness (erythema) and swelling (edema) (OECD, 2021). Skin reactions that have been assessed quantitatively using the Draize scale can be seen in the table 4. Based on table 4, it is proven that the *Averrhoa bilimbi* fruit cream doesn't cause irritation.

c. Human Trial of Anti-aging Cream

The study continued by observing the anti-aging parameters on the use of human hand skin. because no signs of irritation were found in the trial use of anti-aging cosmetics. After using the anti-aging cosmetics for 30 days, the results showed that the cosmetic preparation affected the condition of sebum, moisture, pigmentation, pore diameter, skin elasticity and collagen fibers. There was a decrease in pores and skin pigment after using the anti-aging cream for 30 days and is shown in Figure 2.

Skin Sebum

Sebum is part of the oil found on the surface of the skin. Sebum is produced by the sebaceous glands, or oil glands, and plays a role in helping to keep the skin and hair moist (Jadoon et al., 2015). Its components consist of triglycerides, free fatty acids, wax esters, squalene, cholesterol esters, and cholesterol. Too much sebum can cause body odor. Sebum also has other roles for the skin, including maintaining moisture, skin flexibility, and also

working as a skin protector from bacteria. However, high sebum content will clog pores and mix with various types of dirt. If left continuously, acne will eventually appear (Mahmood et al., 2010).

The skin sebum condition was reduced well after using F1 anti-aging cream compared to F2 and F3. The increase in sebum levels after the use of bases and formulations in F2 and F3 may be due to the oily nature of the W/O emulsion which has a thick oily liquid of stearic acid and glycerin (Rowe et al., 2006). Increased sebum also occurs in the topical cream of *Calendula officinalis* Extract formula due to the W/O emulsion type uses liquid paraffin (Akhtar et al., 2011). The results of the statistical test with paired t test on each concentration (base, F1, F2, F3) with baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin sebum condition (sig>0.05).

Skin Moisture

Moisture barrier is the outer layer of the skin that provides protection to maintain water and moisture in the skin, and protects the skin from external irritants such as dust and bacteria (Akhtar et al., 2011; Jadoon et al., 2015). The moisture barrier layer is made of lipids (oils) that bind skin cells, and when this layer is intact and undisturbed, this layer functions to make your skin feel soft and well-groomed. An example of an intact moisture barrier layer can be seen on baby skin that is supple, smooth, and soft. When the moisture barrier is disturbed or damaged, there will be small, invisible cracks in the skin. Although invisible, through these cracks, skin moisture can escape and disappear, and the danger of irritation can enter the skin more easily. As a result, your skin, which has lost its 'protection', will feel dry, 'tight', and become more vulnerable and sensitive (Sugihartini, 2017).

F3 anti-aging cream is able to increase skin moisture better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. F3 has increased skin moisture by 22,34%. Although F3 has been able to increase skin moisture, statistics show something different. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on skin moisture because sig>0.05. Several studies on cream formulas from extracts have also been able to increase skin moisture, such as in anti-aging potential of a cream containing milk thistle extract. The increase in skin moisture after application of the formulation may be due to the flavonoids from milk thistle because

flavonoids increase the water content in the skin due to swelling of corneocytes on the skin surface (Rasul & Akhtar, 2012). Due to the presence of vitamin E and essential fatty acids, *Sauropus Androgynus* leaf extract may help moisture retention in the epidermis and resistance to dryness (Siallagan et al., 2024). *Averrhoa bilimbi* fruit also contains phenolic and flavonoid content which may also have an effect on increasing skin moisture activity (Rahardhian et al., 2020). With the paired sample t-test it was clear that a significant difference in skin moisture values was produced at week 2 when the base was compared with the formulation (Akhtar et al., 2011).

Skin Pigment

Pigmentation or another name for hyperpigmentation is one of the skin problems where dark spots appear on some areas of the skin. These dark spots cause the overall skin color to become uneven. Skin pigmentation itself can be caused by several reasons. Among them are environmental pollution, UV rays, hormonal problems or other health problems. Hyperpigmentation is a skin condition caused by an increase in melanin, a substance in the body that is responsible for skin coloring (pigment)(Ohguchi et al., 2003). When a person is healthy, their skin color will appear normal. In cases of illness or injury, a person's skin can change color, becoming darker (hyperpigmentation) or lighter (hypopigmentation). Tyrosinase is the key responsible enzyme for synthesis of melanins (Khan et al., 2014; Nur et al., 2017). The reason is that *Averrhoa bilimbi* contains tyrosinase inhibitors, which have skin depigmenting effects (Rahardhian & Suharsanti, 2019).

Comment[L5]: should be eliminated because of double word

Comment[L6]: The reason for what?
The sentence is repaired
Averrhoa bilimbi has a skin depigmentation effect because it contains thyronase inhibitors

F3 anti-aging cream is able to reduce skin pigmentation better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. Although F3 appears to be the most effective in reducing skin pigmentation, results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin pigmentation because sig>0.05. This may be due to the time the cream was applied was not long enough, in other studies the depigmentation effect appeared very well after 8 weeks (Khan et al., 2014).

Comment[L7]: How long does it take?

Skin Pores

Basically, pores are the opening of follicles (gland pockets) to the surface of the skin. These pores function as an exit for oil and hair growth. In other words, pores are small openings in the skin that can pass good skin oil (also known as sebum) and hair to reach the surface of the skin. Pores are said to be large if their size is larger than the average hair

follicle. Some of the impacts experienced when skin pores enlarge include excess oil. The smaller the pore value, the better the skin quality (Aramo, 2012). Conversely, if you have large pores, then the hair or fur that grows on the skin will increase. From the research data above, it is proven that using the cream for 1 month can shrink pores (Sugihartini, 2017).

F3 anti-aging cream is able to reduce the diameter of skin pores better than F1 and F2. Based on figure 2, F3 has the ability to shrink pores by up to 54.18%. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that anti-aging F2 and F3 cream has an effect on the condition of skin pores because sig <0.05. It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have anti-aging activity in shrinking pores.

Skin Elasticity

F3 anti-aging cream is able to improve skin elasticity better than F1 and F2. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that F3 anti-aging cream has an effect on skin elasticity because sig <0.05. One of the characteristics of healthy, fresh and always young skin is having good elasticity. Another characteristic is that when touched, the facial skin feels fresh and supple. The use of F3 cream can increase skin elasticity by up to 16,85% compared to the first day. In other hand, The W/O cream of flower extract of *Calendula officinalis* exhibited aptitude of stimulating skin tightness and improved skin elasticity leading to delayed aging process (Jadoon et al., 2015).

In areas that are often exposed to direct sunlight, changes such as wrinkles and loss of skin elasticity will appear, causing the skin to sag and appear to be pulled downwards, causing the pores to widen (Sugihartini, 2017). Based on the statistical results of the pore data and skin elasticity, there is a match, where F3 has an effect on skin elasticity so that the skin pores also shrink.

Skin Collagen Fibers

Collagen is a type of fibrous and insoluble protein that is most abundant in the human body. Collagen is the main foundation of bones, skin, veins, and ligaments to give them structure and strength. Collagen is also found in many other parts of the body, including blood vessels, corneas and teeth (Jadoon et al., 2015). People who are deficient in collagen can be identified by the appearance of sagging skin, as well as the appearance of fine lines and wrinkles (Young, 2006).

F3 anti-aging cream is able to increase collagen fibers better than F1 and F2. The increase in skin collagen fibers due to F3 was only 1.12%. The results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on increasing skin collagen fibers because sig>0.05.

Conclusion

Based on the anti-aging parameter data that has been presented, anti-aging cream has the potential to reduce skin pores and increase skin elasticity and has been proven to be effective after statistical testing. While for other parameters, *Averrhoa bilimbi* cream with a concentration of 5% (F3) was reported to be the most able to improve skin conditions in a better direction but has not been statistically effective. This can be caused by the application of anti-aging cream only 1 month of use and the skin conditions of each respondent are so diverse that of course this will affect these results.

Acknowledgment

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Table 1. *Averrhoa bilimb*i Fruits Cream Formula

Ingredients	F1(g)	F2(g)	F3 (g)
Extract	1	3	5
Stearic Acid	48	48	48
Cetyl Alcohol	6	6	6
Stearyl alcohol	4	4	4
Nipasol	1	1	1
Nipagin	1	1	1
Glycerin	54	54	54
TEA	4	4	4
Aquadest	Ad	Ad	Ad

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

Table 3. Organoleptic and Homogeneity Test Results of Anti-aging Cream

Formula	Organoleptic			Homogenity
	Smell	**Form**	**Colour**	
Base	*Averrhoa bilimbi* fruit	Semi solid	White	Homogeneous
F1	*Averrhoa bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*Averrhoa bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*Averrhoa bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

Table 4. Results of Irritation Tests on Test Animals and Human

Formula	**Irritation Index**	
	Animal	**Human**
F1	0	0
F2	0	0
F3	0	0
Base	0	0

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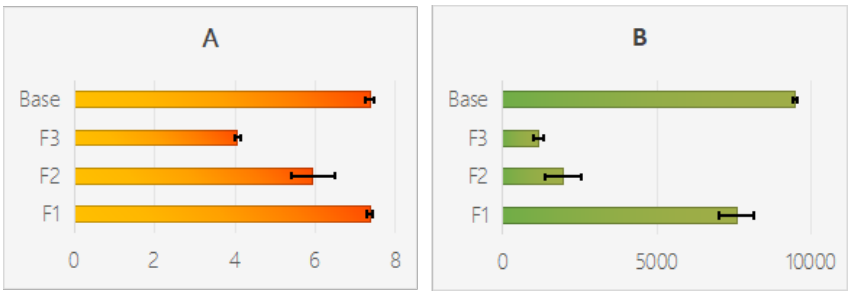

Figure 1. Results of pH (A) and Viscosity (B) tests on anti-aging cream

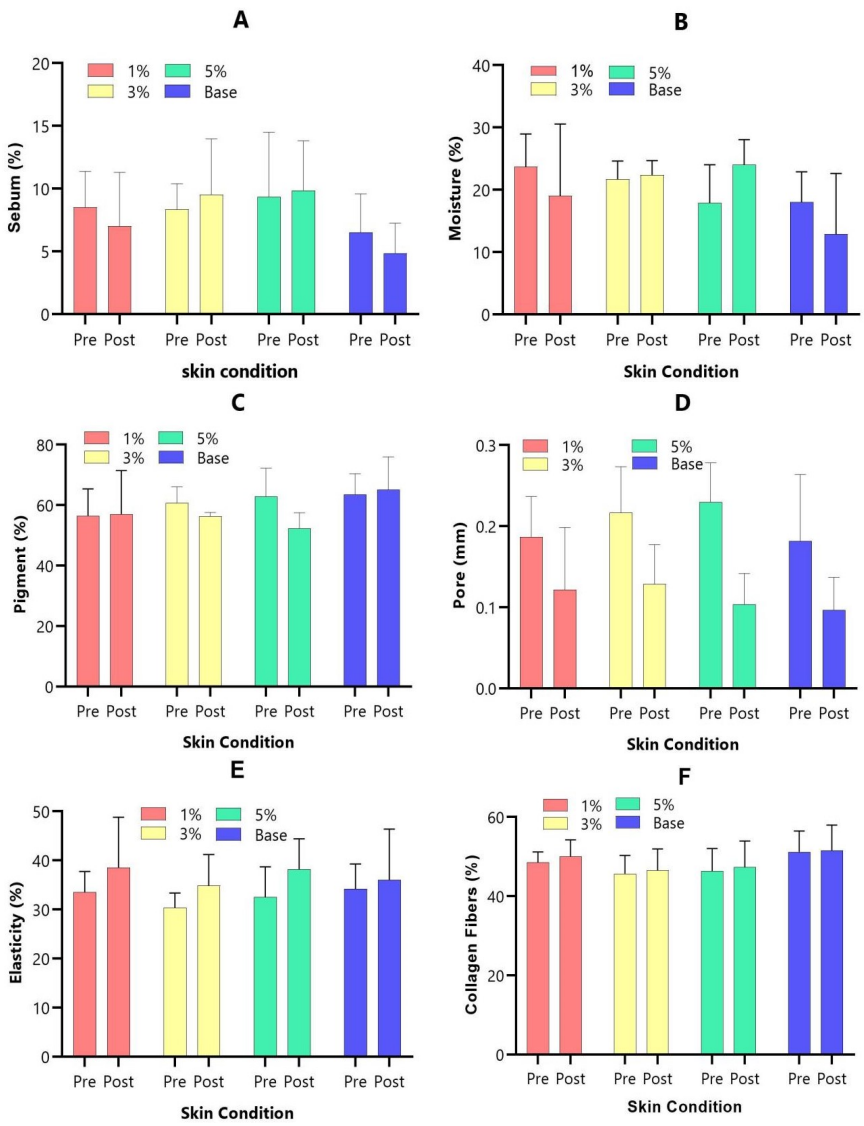

Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use **(A)** Sebum, **(B)** Moisture, **(C)** Pigment, **(D)** Pore, **(E)** Elasticity, **(F)** Collagen Fibers

Anti-aging Potential of *Averrhoa bilimbi* Fruits Cream : a Human Trial Study
Potensi Antipenuaan dari Krim Buah Belimbing Wuluh : Percobaan pada Manusia

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Abstract

Averrhoa bilimbi fruit is one of the native Indonesian plants that has been proven to have antioxidant, sunscreen and tyrosinase enzyme inhibitors activities. Natural chemicals such as polyphenols as flavonoids from natural ingredients can be an alternative anti-aging products. Development of dosage forms in the form of anti-aging cream is needed so that it can be used by the public. The aim of this research is to determine the concentration of *Averrhoa bilimbi* extract that can provide good anti-aging properties. *Averrhoa bilimbi* was extracted with 70% ethanol by remaceration. W/O cream preparation was made with various concentrations of F1 (1%), F2 (3%), and F3 (5%) with *Averrhoa bilimbi* extracts. The cream was tested for characteristics such as organoleptic, homogeneity, pH and viscosity. Irritation test was conducted on animals. All creams were also evaluated for its anti-aging effects with parameters of sebum, moisture, pigment, pore, elasticity, and collagen fibers with skin analyzer on human subjects who had used the cream for 30 days. The results showed that F3 has the best anti-aging effect by increasing moisture, reducing pigmentation, shrinking pores, and increasing skin elasticity. After statistical testing of t-test on each anti-aging parameter, F3 anti-aging cream has been proven to be effective in having the potential to reduce skin pores and increase skin elasticity.
Keywords: *Averrhoa bilimbi*, Cream Formulation, Anti-aging, Human Trial

Abstrak

Buah belimbing wuluh merupakan salah satu tanaman asli indonesia yang telah terbukti memiliki aktivitas antioksidan, tabir surya dan penghambat enzim tirosinase. Zat kimia alami seperti polifenol dan flavonoid dari bahan alam dapat menjadi alternatif produk anti-aging. Pengembangan bentuk sediaan berupa krim anti-aging diperlukan agar dapat digunakan oleh masyarakat. Tujuan dari penelitian ini adalah untuk menentukan konsentrasi ekstrak belimbing wuluh yang dapat memberikan sifat anti-aging yang baik. Belimbing wuluh diekstraksi dengan etanol 70% secara remaserasi. Sediaan krim A/M dibuat dengan berbagai konsentrasi F1 (1%), F2 (3%), dan F3 (5%) dengan ekstrak belimbing wuluh. Krim diuji karakteristiknya seperti organoleptik, homogenitas, pH dan viskositas. Uji iritasi dilakukan pada hewan. Semua krim juga dievaluasi efek anti-penuaannya dengan parameter sebum, kelembaban, pigmen, pori-pori, elastisitas, dan serat kolagen dengan skin analyzer pada subjek manusia yang telah menggunakan krim selama 30 hari. Hasil penelitian menunjukkan bahwa F3 (5%) memiliki efek anti-penuaan terbaik dengan meningkatkan kelembaban, mengurangi pigmentasi, mengecilkan pori-pori, dan meningkatkan elastisitas kulit. Setelah

pengujian statistik t test pada masing-masing parameter anti-aging, krim F3 telah terbukti efektif memiliki potensi untuk memperkecil pori-pori kulit dan meningkatkan elastisitas kulit.
Kata Kunci: *Averrhoa bilimbi*, Formulasi krim, Antipenuaan, Percobaan pada Manusia

Introduction

Skin aging is accelerated by 80% in areas exposed to sunlight (ultraviolet radiation), a process known as photoaging and occurs primarily on the face, neck, hands, and lower arms and legs (Aramo, 2012). Photoaging occurs due to a combination of short wavelengths (UVB) that hit the outer layer of the skin (epidermis) and long wavelengths (UVA) to the middle layer (dermis) (Binic et al., 2013). This exposure results in direct damage to the skin cell nucleus in the formation of reactive oxygen species (ROS) and other free radicals and reacts with important molecules in connective tissue and cell membranes. Clinically, acute UV-induced skin damage is seen as erythema, edema, and blistering of the sunburned skin followed by peeling, then tanning (a sign of long-term damage). Characteristics of skin aging for Asian skin types are a decrease in sebum levels, increased pigment and wrinkles in the skin, and loss of skin moisture (Dayan, 2008).

Cosmetic products have one of the benefits to protect the skin because of the ingredients they contain, so they can affect the biological function of the skin. Some plant extracts and antioxidants obtained from natural sources are able to prevent aging and can improve skin health. To prevent skin from aging or wrinkles, natural phytochemical sources are preferred. Plant extracts rich in secondary metabolites, such as flavonoids, phenolic acids, saponins and alkaloids, which have collagen synthesis activity, are being widely used for the development of topical anti-aging skin cosmetic products (Suharsanti et al., 2019). One of the natural sources that contains phenolic and flavonoids compounds is *Averrhoa bilimbi* fruit. So it is very promising to make *Averrhoa bilimbi* fruit as a cosmetic product.

The total phenolic content of *Averrhoa bilimbi* fruits extract is 19.8022 ± 1.6696 mg GAE/g more than leaves ethanolic extract 6.5886±0.0590 mg GAE/g and total flavonoids 24.7458 ± 0.3291 mg RE/g more than leaves ethanolic extract 13.3126±0.5695 mg RE/g. The SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can be described in the category of sunblock assessment with maximum protection. *Averrhoa bilimbi* fruit extract has an IC_{50} value of antioxidant activity of 2.33 ± 0.33 mg/ml and tyrosinase inhibitor activity with an IC_{50} value of 186.85 ± 9.37 mg/mL (Suharsanti et al., 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa*

bilimbi fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has an IC_{50} value of 74,625 ppm which is included in the category of strong antioxidants (Kusuma et al., 2023). In other research, The test results of antioxidant activity with the DPPH method using the most effective UV-Vis spectrophotometer as the antioxidant cream of the *Averrhoa bilimbi* leaves extract has a value of IC50 0.118 ppm and vitamin C as comparator has a value of IC_{50} 0.0327 ppm (Ariem et al., 2020). Shooting gel preparation with a combination of *Aloe vera* and *Averrhoa bilimbi* fruits formula I (85%) can increase the moisture value of human skin between before and after use (Ariani & Suharsanti, 2018a). Cream containing of 2% *Averrhoa bilimbi* leaf extract () has favorable physical characteristics and effectively heals incision wounds in male white mice (Suena et al., 2024). The irritation test showed that all concentrations of the shooting gel preparation formula didn't cause irritation to the skin of mice (Ariani & Suharsanti, 2018b).

Based on this, *Averrhoa bilimbi* fruit which has been proven to contain efficacious compounds and has activity as a skin whitener in vitro needs to be developed into an anti-aging skin cosmetic preparation. Anti-aging cream has a comfortable texture when applied to the skin and has good aesthetic value, so it can improve the skin care experience. When compared to other dosage forms, anti-aging cream is non-sticky and easier to clean and apply.

Methods

a. Material

Averrhoa bilimbi dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin, stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor, Indonesia), New Zealand Rabbits aged 12-16 weeks, Voluneteers include subjects aged 19–25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State), skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

b. Extraction of *Averrhoa bilimbi*

The 200 g dried fruits powdered were remaserated with 70% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract (Rahardhian et al., 2019).

c. Formula Preparation

The extract obtained was made into cream preparations with concentrations of 1, 3, and 5% (Iskandar et al., 2016). The formula preparation of *Averrhoa bilimbi* extract can be seen in table 1.

Averrhoa bilimbi Fruits extract cream preparation is made by melting method. Water-soluble ingredients consisting of TEA, nipagin, glycerin are mixed and heated in distilled water at a temperature of 70°C. The oil phase consisting of stearic acid, cetyl alcohol, stearyl alcohol, and nipasol is heated at the same temperature. After that, the water phase is added little by little into the oil phase mixture while stirring until a homogeneous cream mass is formed. After that, the extract is added and mixed until homogeneous.

d. Physical Characteristics of Anti-aging Cream

Organoleptic Test

This examination is carried out by visually viewing the preparation including form, color and odor. This aims to determine the visual physical properties of cream (Ariem et al., 2020)

Homogeneity Test

This examination is carried out by placing the preparation between two glass objects and then observing it. The purpose of this test is to determine the mixing between each particle of the preparation made. The requirements for this test are that there are no visible coarse particles and they are perfectly homogeneously mixed (Ariem et al., 2020).

pH Test

Determination of the pH of the preparation is done using a digital pH meter solution. A good clay stick has a pH of 4.5-6.5 which is the ideal pH for topical preparations. This is in line with the pH of the skin which ranges from 4.5-6.5 (Ariem et al., 2020).

Viscosity Test

Determination of the viscosity of the cream preparation was carried out using a digital brookfield viscometer using the R7 spindle and by knowing the changes in viscosity in each cream formula. Reading the viscosity results in Cp (Ramdhan & Yusuf, 2023).

e. Ethical Clearance

Ethical clearance is a written ethical eligibility issued by the Medical/Health Research Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang with the number No. 313/V/2019/Bioethics Commission after meeting the established requirements. The creation of ethical clearance is necessary because this study uses living subjects, test animals and humans, so it is necessary to ensure that this study has met the

principle of respecting animal and human dignity. Ethical approval includes animal and human testing for irritation tests as well as antiaging tests on humans.

f. Irritation Test on test animals

Irritation Test was conducted with male new zealand rabbits aged 12-16 weeks with the OECD method. The fur of the back and sides was shaved carefully ± 24 hours before application. The area is about 6 cm^2. Apply 0.5 g of cream to each formula. Wrap the area with a semi-occlusive bandage (half-closed, but still allows air exchange). Observe the animal after 1 hour, 24 hours, 48 hours, and 72 hours directly to the open skin. Irritation tests were also conducted on humans with the same treatment. Observe the presence of erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring System) according to table 2. Calculate the average score for each parameter at 24, 48, and 72 hours (OECD, 2021). Irritation conditions were also observed during application of the cream to humans during the initial 3 days of use.

g. Informed Consent

In this study, the subject was asked to sign an informed consent. The informed consent statement contains confirmation from the research subject that the person understands the research process, and rights including the right to refuse or withdraw from the study without any negative consequences, and an understanding that participation in the study is voluntary. Inclusion criteria include subjects aged 19–25 years, male or female, subjects not consuming skin vitamins or mineral supplements, all subjects in good condition based on health history. Exclusion criteria are subjects with degenerative diseases, skin diseases, inflammatory diseases, or consuming drugs, antioxidants, vitamins, or health supplements, subjects who are pregnant or breastfeeding. Signature of the research subject, date, place, and others if needed.

h. Human Trial of Anti-aging Cream

Anti-aging testing of *Averrhoa bilimbi* Fruits Cream preparation was conducted for 30 days with 24 volunteer. On the first day, the volunteer's skin was checked using a skin analyzer performed by a doctor at the Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia) to determine the skin condition before treatment, after 30 days it was checked again using a skin analyzer. For 30 days, the cream was used on the back of the volunteer's hand (according to the formula group) every morning and evening. Checks by the clinic's doctor included aging parameters such as sebum, moisture, pigment, pore, elasticity, and

collagen fibers. To control the volunteer's compliance, every day the volunteer was required to check their skin with a portable skin analyzer that can check the water content and oil content of the volunteer.

i. Analysis Data

The results of each skin parameter before and after using the cream for 30 days were statistically tested using a paired t-test at each concentration. From the three formulas, one formula was chosen that had the best anti-aging effect.

Results and Discussion

a. Characteristics of Anti-aging Cream

Anti-aging cream preparations F1, F2, and F3 are semi-solid, have a fragrant odor, and have colors ranging from pale yellow to deep yellow. Anti-aging cream preparations F1, F2, and F3 are homogeneous. The results of organoleptic and homogeneity tests can be seen in table 3.

The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range. The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus et al., 2024). The appropriate viscosity will help the cream to be easily applied, spread evenly on the skin, and increase the absorption of active substances. So all cream preparation formulas have met the pH and viscosity quality requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

b. Irritation Test of Anti-aging Cream

The results of the irritation test on test animals using rabbits and human hand skin after 72 hours did not find any signs of irritation such as redness (erythema) and swelling (edema) (OECD, 2021). Skin reactions that have been assessed quantitatively using the Draize scale can be seen in the table 4. Based on table 4, it is proven that the *Averrhoa bilimbi* fruit cream doesn't cause irritation.

c. Human Trial of Anti-aging Cream

The study continued by observing the anti-aging parameters on the use of human hand skin. because no signs of irritation were found in the trial use of anti-aging cosmetics.

After using the anti-aging cosmetics for 30 days, the results showed that the cosmetic preparation affected the condition of sebum, moisture, pigmentation, pore diameter, skin elasticity and collagen fibers. There was a decrease in pores and skin pigment after using the anti-aging cream for 30 days and is shown in Figure 2.

Skin Sebum

Sebum is part of the oil found on the surface of the skin. Sebum is produced by the sebaceous glands, or oil glands, and plays a role in helping to keep the skin and hair moist (Jadoon et al., 2015). Its components consist of triglycerides, free fatty acids, wax esters, squalene, cholesterol esters, and cholesterol. Too much sebum can cause body odor. Sebum also has other roles for the skin, including maintaining moisture, skin flexibility, and also working as a skin protector from bacteria. However, high sebum content will clog pores and mix with various types of dirt. If left continuously, acne will eventually appear (Mahmood et al., 2010).

The skin sebum condition was reduced well after using F1 anti-aging cream compared to F2 and F3. The increase in sebum levels after the use of bases and formulations in F2 and F3 may be due to the oily nature of the W/O emulsion which has a thick oily liquid of stearic acid and glycerin (Rowe et al., 2006). In F2 and F3 the amount of extract increases so that the amount of water in the preparation decreases, making it more oily. Increased sebum also occurs in the topical cream of *Calendula officinalis* Extract formula due to the W/O emulsion type uses liquid paraffin (Akhtar et al., 2011). The results of the statistical test with paired t test on each concentration (base, F1, F2, F3) with baseline (skin condition before being given anti-aging cream) didn't show any effect (not different) of using anti-aging cream on skin sebum condition (sig>0.05) . The use of creams F1, F2, and F3 was shown to have no statistical impact, perhaps the duration of use was not long enough or it took more than 30 days to see an impact on skin sebum parameters.

Skin Moisture

Moisture barrier is the outer layer of the skin that provides protection to maintain water and moisture in the skin, and protects the skin from external irritants such as dust and bacteria (Akhtar et al., 2011; Jadoon et al., 2015). The moisture barrier layer is made of lipids (oils) that bind skin cells, and when this layer is intact and undisturbed, this layer functions to make your skin feel soft and well-groomed. An example of an intact moisture barrier layer can be seen on baby skin that is supple, smooth, and soft. When the moisture

barrier is disturbed or damaged, there will be small, invisible cracks in the skin. Although invisible, through these cracks, skin moisture can escape and disappear, and the danger of irritation can enter the skin more easily. As a result, your skin, which has lost its 'protection', will feel dry, 'tight', and become more vulnerable and sensitive (Sugihartini, 2017).

F3 anti-aging cream is able to increase skin moisture better than F1 and F2. F3 has increased skin moisture by 22,34%. Although F3 has been able to increase skin moisture, statistics show something different. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on skin moisture because sig>0.05 (not different). The use of creams F1, F2, and F3 was shown to have no statistical impact, perhaps the duration of use was not long enough or it took more than 30 days to see an impact on moisture parameters. The use of anti-aging cream with a higher concentration of moringa leaf extract of 9% for 14 days also did not provide a significant effect on skin moisture conditions (Sugihartini, 2017). Several studies on cream formulas from extracts have also been able to increase skin moisture, such as in anti-aging potential of a cream containing milk thistle extract. The increase in skin moisture after application of the formulation may be due to the flavonoids from milk thistle because flavonoids increase the water content in the skin due to swelling of corneocytes on the skin surface (Rasul & Akhtar, 2012). Due to the presence of vitamin E and essential fatty acids, *Sauropus Androgynus* leaf extract may help moisture retention in the epidermis and resistance to dryness (Siallagan et al., 2024). *Averrhoa bilimbi* fruit also contains phenolic and flavonoid content which may also have an effect on increasing skin moisture activity (Rahardhian et al., 2020). With the paired sample t-test it was clear that a significant difference in skin moisture values was produced at week 2 when the base was compared with the formulation (Akhtar et al., 2011).

Skin Pigment

Pigmentation or another name for hyperpigmentation is one of the skin problems where dark spots appear on some areas of the skin. These dark spots cause the overall skin color to become uneven. Skin pigmentation itself can be caused by several reasons. Among them are environmental pollution, UV rays, hormonal or other health problems. Hyperpigmentation is a skin condition caused by an increase in melanin, a substance in the body that is responsible for skin coloring (pigment)(Ohguchi et al., 2003). When a person is healthy, their skin color will appear normal. In cases of illness or injury, a person's skin can

change color, becoming darker (hyperpigmentation) or lighter (hypopigmentation). Tyrosinase is the key responsible enzyme for synthesis of melanins (Khan et al., 2014; Nur et al., 2017). *Averrhoa bilimbi* has a skin depigmentation effect because it contains thyronase inhibitors (Rahardhian & Suharsanti, 2019).

F3 anti-aging cream is able to reduce skin pigmentation better than F1 and F2. F3 can reduce skin pigmentation by 15.08% better than F1 and F2. Although F3 appears to be the most effective in reducing skin pigmentation, results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) didn't show any effect of using anti-aging cream on skin pigmentation because sig>0.05 (not different). The use of Moringa leaf extract (3%, 6%, and 9%) cream for 15 days in each formula was able to reduce the skin spot value, but the skin spot value before and after the use of Moringa leaf extract cream didn't differ significantly. This may be due to the time the cream was applied was not long enough, at least 2 months (8 weeks). In a similar study, the depigmentation effect appeared very good after 8 weeks. (Khan et al., 2014).

Skin Pores

Basically, pores are the opening of follicles (gland pockets) to the surface of the skin. These pores function as an exit for oil and hair growth. In other words, pores are small openings in the skin that can pass good skin oil (also known as sebum) and hair to reach the surface of the skin. Pores are said to be large if their size is larger than the average hair follicle. Some of the impacts experienced when skin pores enlarge include excess oil. The smaller the pore value, the better the skin quality (Aramo, 2012). Conversely, if you have large pores, then the hair or fur that grows on the skin will increase. From the research data above, it is proven that using the cream for 1 month can shrink pores (Sugihartini, 2017).

F3 anti-aging cream is able to reduce the diameter of skin pores better than F1 and F2. Based on figure 2, F3 has the ability to shrink pores by up to 54.18%. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that anti-aging F2 and F3 cream has an effect on the condition of skin pores because sig <0.05 (different). This means that the concentration of the extract has an effect on skin pores. It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have anti-aging activity in shrinking pores. Tannin compounds which are included in phenolics can be used as astringents. Astringents in plants are useful for shrinking pores (Puspitasari, 2011).

Skin Elasticity

F3 anti-aging cream is able to improve skin elasticity better than F1 and F2. The results of statistical tests with paired t-tests on each concentration with baseline (skin condition before being given anti-aging cream) show that F3 anti-aging cream has an effect on skin elasticity because sig <0.05 (different). This means that the concentration of the extract has an effect on skin elasticity. One of the characteristics of healthy, fresh and always young skin is having good elasticity. Another characteristic is that when touched, the facial skin feels fresh and supple. The use of F3 cream can increase skin elasticity by up to 16,85% compared to the first day. In other hand, The W/O cream of flower extract of *Calendula officinalis* exhibited aptitude of stimulating skin tightness and improved skin elasticity leading to delayed aging process (Jadoon et al., 2015).

In areas that are often exposed to direct sunlight, changes such as wrinkles and loss of skin elasticity will appear, causing the skin to sag and appear to be pulled downwards, causing the pores to widen (Sugihartini, 2017). Tannin compounds which are included in phenolics can be used as astringents. Astringents in plants are useful for shrinking pores (Puspitasari, 2011). If the pores shrink then the skin elasticity increases. Based on the statistical results of the pores and skin elasticity data, there is a match where F1, F2, and F3 affect skin elasticity so that the skin pores also shrink. F3 has better activity than F1 and F2 because the extract concentration is higher.

Skin Collagen Fibers

Collagen is a type of fibrous and insoluble protein that is most abundant in the human body. Collagen is the main foundation of bones, skin, veins, and ligaments to give them structure and strength. Collagen is also found in many other parts of the body, including blood vessels, corneas and teeth (Jadoon et al., 2015). People who are deficient in collagen can be identified by the appearance of sagging skin, as well as the appearance of fine lines and wrinkles (Young, 2006).

F3 anti-aging cream is able to increase collagen fibers better than F1 and F2. The increase in skin collagen fibers due to F3 was only 1.12%. The results of the statistical test with a paired t test on each concentration with the baseline (skin condition before being given anti-aging cream) did not show any effect of using anti-aging cream on increasing skin collagen fibers because sig>0.05 (not different).

Conclusion

Based on the anti-aging parameter data that has been presented, anti-aging cream has the potential to reduce skin pores and increase skin elasticity and has been proven to be effective after statistical testing. While for other parameters, *Averrhoa bilimbi* cream with a concentration of 5% (F3) was reported to be the most able to improve skin conditions in a better direction but has not been statistically effective. This can be caused by the application of anti-aging cream only 1 month of use and the skin conditions of each respondent are so diverse that of course this will affect these results. The concentration of the extract needs to be increased to obtain a more impactful effect on skin aging parameters.

Acknowledgment

This research was supported by Direktorat Riset dan Pengabdian Masyarakat (DRPM) from Kementerian Ristek Dikti through the "PKPT (Program Kerjasama Perguruan Tinggi)" program.

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Table 1. *Averrhoa bilimb*i Fruits Cream Formula

Ingredients	F1(g)	F2(g)	F3 (g)
Extract	1	3	5
Stearic Acid	48	48	48
Cetyl Alcohol	6	6	6
Stearyl alcohol	4	4	4
Nipasol	1	1	1
Nipagin	1	1	1
Glycerin	54	54	54
TEA	4	4	4
Aquadest	Ad	Ad	Ad

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

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448

449 **Table 3.** Organoleptic and Homogeneity Test Results of Anti-aging Cream

Formula	Organoleptic			Homogenity
	Smell	**Form**	**Colour**	
Base	*Averrhoa bilimbi* fruit	Semi solid	White	Homogeneous
F1	*Averrhoa bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*Averrhoa bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*Averrhoa bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

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451 **Table 4.** Results of Irritation Tests on Test Animals and Human

Formula	**Irritation Index**	
	Animal	**Human**
F1	0	0
F2	0	0
F3	0	0
Base	0	0

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Figure 1. Results of pH **(A)** and Viscosity **(B)** tests on anti-aging cream

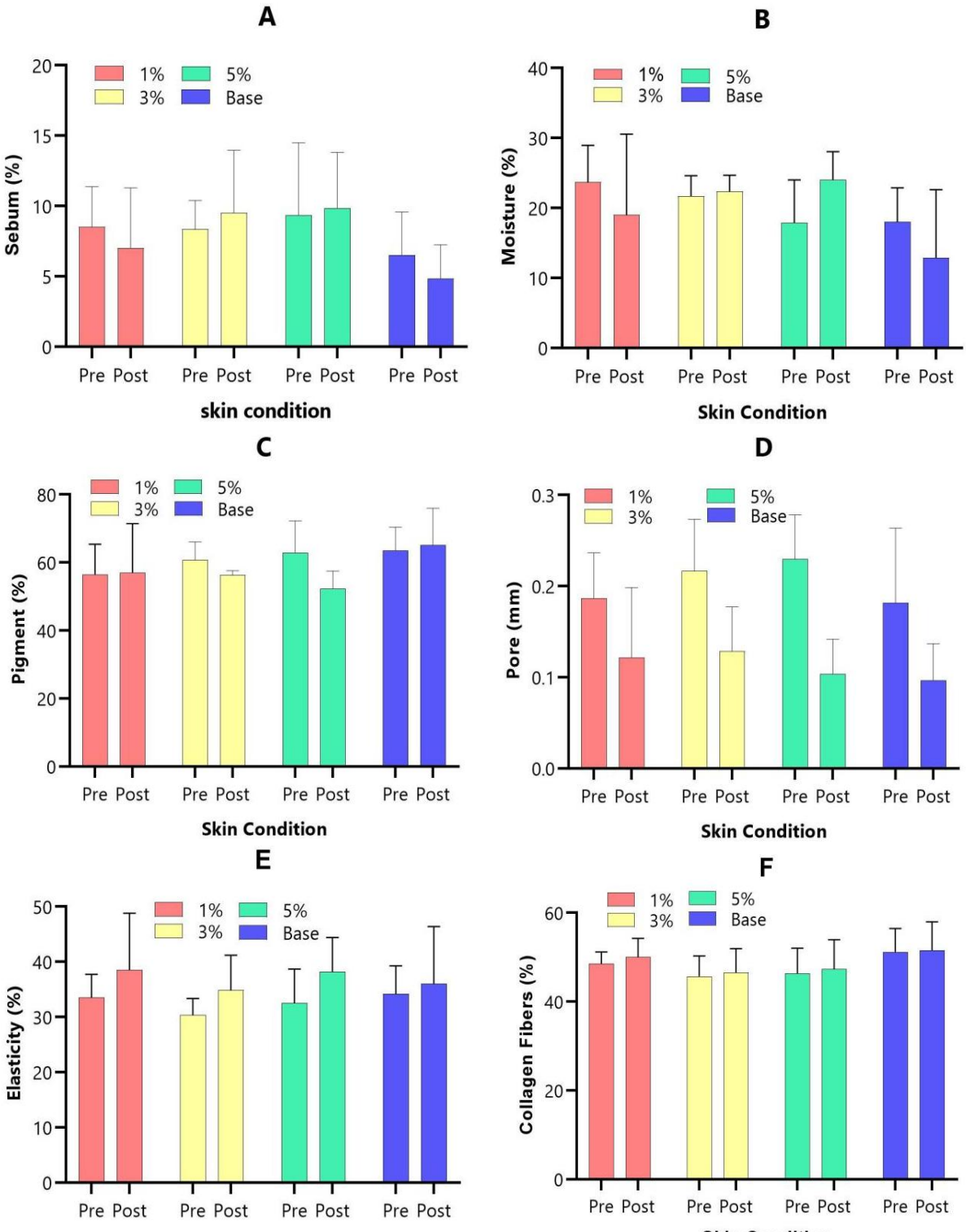

Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use **(A)** Sebum, **(B)** Moisture, **(C)** Pigment, **(D)** Pore, **(E)** Elasticity, **(F)** Collagen Fibers

Author's Response Letter to Editor and Reviewers Comments

Journal name : Jurnal Jamu Indonesia (JJI)

Manuscript Title : *Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study*

Manuscript No : JJI-401

Type of paper : Original Article

Authors Name : Ririn Suharsanti*, Muhammad Ryan Radix Rahadhian, Nining Sugihartini, Endang Lukitaningsih

Reply to the editor and reviewers' comments

Editor or Reviewer Number	Original comments of the editor/reviewer	Reply by the author(s)	Changes done on page number and line number
Editor	Reduce the similarity index of the manuscript to below 25%, as currently it stands at 55% (see attached file).	we have deleted the turnitin repository of the institution related to our previous article, so that it is not detected as autoplagiarism of the article when other parties check we have also attached the latest turnitin results report for our article	Attached in the revision section
3411	What is the correct writing?	we rewrite as suggested we have fixed it with the correct word : **Photoaging**	Page 2, line 49 Page 2, line 50
3411	Samples used by dried fruit or powder?	we rewrite as suggested we write according to what we use for the sample: The 200 g **dried fruit powdered**	Page 3, line 102

Editor or Reviewer Number	Original comments of the editor/reviewer	Reply by the author(s)	Changes done on page number and line number
		were remaserated with 70% ethanol….ect	
3411	Reference articles on making extract concentrations in creams please cite	we rewrite as suggested we have added the citation: **Iskandar et al., 2016**	Page 3, line 107
3411	Should be eliminated because of double word	we rewrite as suggested we have replaced with : **hormonal or other health problems**	Page 8, line 262
3411	The reason for what? The sentence is repaired Averrhoa bilimbi has a skin depigmentation effect because it contains thyronase inhibitors	we have changed the sentence as suggested : **Averrhoa bilimbi has a skin depigmentation effect because it contains thyronase inhibitors**	Page 8, line 268-269
3411	How long does it take?	we have added the length of time that may be required for anti-aging studies referring to similar research **This may be due to the time the cream was applied was not long enough, at least 2 months (8 weeks). In a similar study, the depigmentation effect appeared very good after 8 weeks.**	Page 8, line 277-279
3412	Are there any gender criteria in both animals and humans? If it is not needed, it can be explained in the method or discussion	We have written as suggested In animals: **Irritation Test was conducted with male new zealand rabbits aged 12-16 weeks with the OECD method** In Human: the same as the volunteer requirements for anti-aging tests stated in the informed consent (in the inclusion criteria section)	Page 4, line 141-142

Editor or Reviewer Number	Original comments of the editor/reviewer	Reply by the author(s)	Changes done on page number and line number
		Inclusion criteria include subjects aged 19–25 years, male or female, subjects not consuming skin vitamins or mineral supplements, all subjects in good condition based on health history. .	Page 5, line 156-157
3412	Please add to the discussion the relationship between the characterization of the preparation and the test results on animals and humans	We have written as suggested we have written the relationship of pH and viscosity characteristic tests to skin irritation and anti-aging effect pH : **The increase in pH value occurs as the extract increases but is still within a safe pH range. The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al., 2020; SNI, 1996).** Viscosity : **Viscosity also increases with increasing extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus et al., 2024). The appropriate viscosity will help the cream to be easily applied, spread evenly on the skin, and increase the absorption of active substances.**	Page 6, line184-186 Page 6, line186-190

Note : Highlighted yellow for changes to response reviewer.

Editor's comment

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 is an Indonesian native plant known for its 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.
Keywords: *Averrhoa bilimbi*, anti-aging cream, herbal cosmetics, clinical trial, skin elasticity, pore size

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.

Editor's comment

Kata Kunci: Belimbing wuluh*, Averrhoa bilimbi,* krim antipenuaan, uji klinis, elastisitas kulit, pori-pori

Introduction

Skin aging is a complex biological process, with approximately 80% of visible signs attributed to ultraviolet (UV) exposure, a condition referred to as photoaging. This phenomenon primarily affects sun-exposed areas such as the face, neck, hands, and lower limbs (Aramo, 2012). UVB rays affect the epidermis, while UVA rays penetrate deeper into the dermis, inducing the formation of reactive oxygen species (ROS) and free radicals that damage key structural proteins in the skin, such as collagen and elastin (Binic dkk., 2013).

In Asian skin types, aging is characterized by reduced sebum production, increased pigmentation and wrinkling, and diminished moisture retention (Dayan, 2008) . Topical cosmetics containing bioactive natural compounds are increasingly favored for their dual roles in preventing skin aging and maintaining skin health. Phytochemicals—such as flavonoids, phenolic acids, saponins, and alkaloids—are known to stimulate collagen synthesis and exert antioxidant effects, making them valuable for the development of anti-aging skincare products (Suharsanti dkk., 2019).

Averrhoa bilimbi, a plant native to Indonesia, has shown potential in this regard. The fruit of *A. bilimbi* contains higher levels of phenolics and flavonoids compared to its leaves, with total phenolic content reaching 19.80 ± 1.67 mg GAE/g and total flavonoid content of 24.75 ± 0.33 mg RE/g (Suharsanti dkk., 2019) . In addition, the fruit extract demonstrates sun protection factor (SPF) values indicative of maximum protection at 300 ppm concentration, antioxidant activity with an IC_{50} of 2.33 ± 0.33 mg/mL, and tyrosinase inhibitory activity with an IC_{50} of 186.85 ± 9.37 mg/mL.

Previous studies on *A. bilimbi* have primarily focused on wound-healing applications using leaf extracts, while the fruit extract remains underutilized, particularly in topical anti-aging formulations. Creams are a preferred topical dosage form due to their ease of application, favorable aesthetics, and comfort on the skin.

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

Editor's comment

Materials and Methods

Materials

Dried *Averrhoa 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).

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 dkk., 2019).

Cream Formulation

Creams containing *A. bilimbi* extract were prepared in concentrations of 1% (F1), 3% (F2), and 5% (F3). The formulation method referred to Iskandar et al. (Iskandar dkk., 2016). The composition of each cream formula is presented in **Table 1**. The water-soluble phase—comprising TEA, nipagin, and glycerin dissolved in distilled water—was heated to 70°C. Simultaneously, the oil phase—comprising stearic acid, cetyl alcohol, stearyl alcohol, and nipasol—was also heated to the same temperature. The aqueous phase was then gradually added to the oil phase under continuous stirring until a stable emulsion formed. Once the emulsion was uniform and slightly cooled, the extract was added and mixed thoroughly until homogeneous.

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

Ingredient	F1 (%)	F2 (%)	F3 (%)
A. bilimbi extract	1	3	5
Stearic Acid	48	48	48
Cetyl Alcohol	6	6	6
Stearyl alcohol	4	4	4
Nipasol	1	1	1

Comment[WN1]: Cek yang benar, % atau g? Harusnya % bukan

Editor's comment

Nipagin	1	1	1
Glycerin	54	54	54
TEA	4	4	4
Distilled water	ad 100	ad 100	ad 100

Note: "ad 100" indicates addition of distilled water to make the total quantity 100%.

Evaluation of Cream Characteristics

Organoleptic evaluation included visual observation of the cream's form, color, and odor. Homogeneity was assessed by spreading a small portion of the cream between two glass slides and checking for uniformity and absence of coarse particles. The pH was determined using a digital pH meter to ensure it remained within the optimal topical range of 4.5–6.5. Viscosity was measured using a Brookfield viscometer with R7 spindle and recorded in centipoise (cP) units (Ramdhan & Yusuf, 2023).

Ethical Clearance

Ethical approval for the study was granted by the Bioethics Commission for Medical and Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang, with approval number 313/V/2019/Bioethics Commission. The ethical clearance covered both animal testing and human trials, ensuring full compliance with ethical standards.

Irritation Test

The irritation test was conducted in accordance with OECD guidelines (OECD, 2021). On each rabbit, a 6 cm^2 area of shaved dorsal skin was treated with 0.5 g of cream and covered using a semi-occlusive bandage. Observations for erythema and edema were made at 1, 24, 48, and 72 hours, and results were scored using the Draize method (**Table 2**). The same procedure was applied to human subjects, with additional daily observation during the first three days of product use.

Table 2. Irritation Score (Draize Scoring System)

Condition	Score
There isn't any	0
Very Light	1
Clearly Visible	2

Editor's comment

Heavy	3
Very heavy	4

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.

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.

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.

Results and Discussion

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**.

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

Formula	Smell	Form	Color	Homogeneity
Base	*A. bilimbi* fruit	Semi solid	White	Homogeneous

Editor's comment

F1	*A. bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*A. bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*A. bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

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.

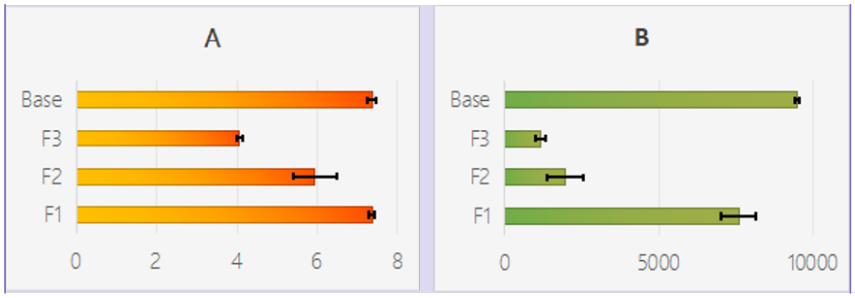

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Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

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

Human Trial: Anti-Aging Parameters

Editor's comment

Anti-aging efficacy was assessed by comparing skin parameters before and after 30 days of cream application. The skin condition improvements are visualized in Figure 2.

Sebum levels slightly increased in F2 and F3 groups, likely due to the higher oil content in W/O formulations. However, these changes were statistically non-significant ($p > 0.05$). Excessive sebum can obstruct pores, but the increase here did not exceed normal levels and may be counteracted by the formula's other active properties.

F3 showed the highest increase in skin hydration (22.34%) after 30 days of application. Nonetheless, this improvement was not statistically significant ($p > 0.05$). Moisturizing effects of flavonoid-rich extracts have been documented, but a longer duration may be needed to observe significant change (Rasul & Akhtar, 2012; Sugihartini, 2017).

F3 also exhibited the most effective pigmentation reduction (15.08%). However, statistical analysis did not support significance ($p > 0.05$). This result aligns with previous studies that reported visible depigmentation only after 8 weeks of application (Khan dkk., 2014).

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Comment[WN3]: Informasi t-test belum disajikan di gambar ini, mohon dilengkapi! Kemudian gunakan F1, F2, F3 dan base seperti sebelumnya! Mohon data excelnya dikirimkan juga!

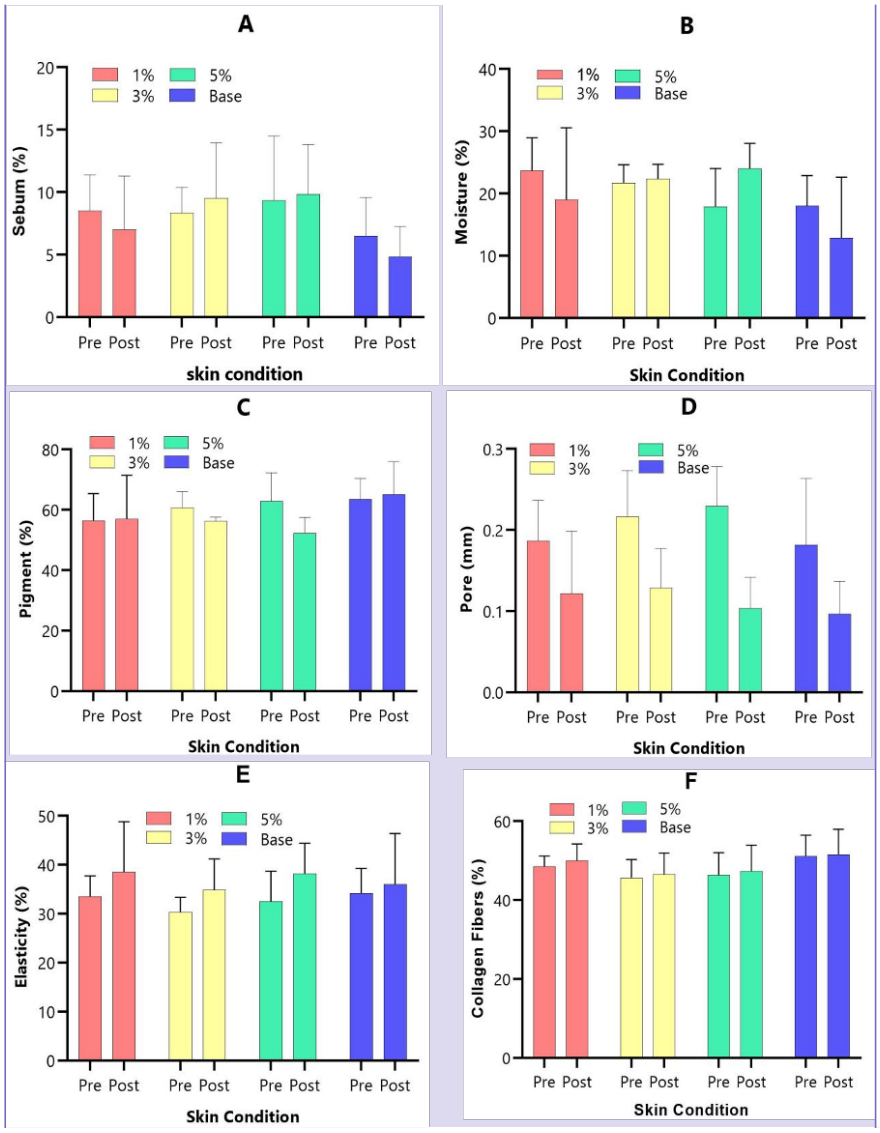

Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers

Significant reduction in pore diameter was observed in both F2 and F3 groups ($p < 0.05$), with F3 achieving the greatest reduction (54.18%). This effect may be attributed to

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phenolic compounds, including tannins, which act as astringents and help tighten skin pores (Puspitasari, 2011).

Skin elasticity improved significantly in the F3 group ($p < 0.05$), with an increase of 16.85%. This result suggests that *A. bilimbi* extract may contribute to collagen preservation or synthesis. Improved elasticity correlates well with the observed pore shrinkage, indicating restored dermal integrity (Jadoon dkk., 2015).

Collagen fiber density showed a minimal increase (1.12%) in the F3 group and was not statistically significant ($p > 0.05$). Collagen improvement may require prolonged use or higher extract concentrations.

Among the three cream formulations tested, F3 (5% *A. bilimbi* extract) demonstrated the most consistent improvements across multiple skin-aging parameters. Statistically significant changes were observed in pore size and elasticity, suggesting strong anti-aging potential. Although other parameters did not show statistical significance, trends in moisture and pigmentation improvement were evident. The results indicate that *A. bilimbi* extract is a promising active ingredient for anti-aging cosmetic formulations, particularly in W/O cream form.

Conclusion

Based on the findings from this study, the cream formulation containing 5% *Averrhoa 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 other parameters such as moisture content, pigmentation, and collagen fiber density showed positive trends, they did not reach statistical significance within the 30-day application period. The results support the use of *A. bilimbi* fruit extract in topical formulations for anti-aging purposes. Further studies with longer treatment durations and higher extract concentrations are recommended to strengthen and expand these findings.

Acknowledgment

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through the Inter-University Cooperation Program (Program Kerja Sama Perguruan Tinggi/PKPT). We also thank the volunteers and medical personnel at Rista Clinic Beauty Care, Demak, for their contribution to the clinical aspects of this study.

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Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use **(A)** Sebum, **(B)** Moisture, **(C)** Pigment, **(D)** Pore, **(E)** Elasticity, **(F)** Collagen Fibers

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 is an Indonesian native plant known for its 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.
Keywords: *Averrhoa bilimbi*, anti-aging cream, herbal cosmetics, clinical trial, skin elasticity, pore size

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.

Kata Kunci: Belimbing wuluh*, Averrhoa bilimbi,* krim antipenuaan, uji klinis, elastisitas kulit, pori-pori

Introduction

Skin aging is a complex biological process, with approximately 80% of visible signs attributed to ultraviolet (UV) exposure, a condition referred to as photoaging. This phenomenon primarily affects sun-exposed areas such as the face, neck, hands, and lower limbs (Aramo, 2012). UVB rays affect the epidermis, while UVA rays penetrate deeper into the dermis, inducing the formation of reactive oxygen species (ROS) and free radicals that damage key structural proteins in the skin, such as collagen and elastin (Binic dkk., 2013).

In Asian skin types, aging is characterized by reduced sebum production, increased pigmentation and wrinkling, and diminished moisture retention (Dayan, 2008) . Topical cosmetics containing bioactive natural compounds are increasingly favored for their dual roles in preventing skin aging and maintaining skin health. Phytochemicals—such as flavonoids, phenolic acids, saponins, and alkaloids—are known to stimulate collagen synthesis and exert antioxidant effects, making them valuable for the development of anti-aging skincare products (Suharsanti dkk., 2019).

Averrhoa bilimbi, a plant native to Indonesia, has shown potential in this regard. The fruit of *A. bilimbi* contains higher levels of phenolics and flavonoids compared to its leaves, with total phenolic content reaching 19.80 ± 1.67 mg GAE/g and total flavonoid content of 24.75 ± 0.33 mg RE/g (Suharsanti dkk., 2019) . In addition, the fruit extract demonstrates sun protection factor (SPF) values indicative of maximum protection at 300 ppm concentration, antioxidant activity with an IC_{50} of 2.33 ± 0.33 mg/mL, and tyrosinase inhibitory activity with an IC_{50} of 186.85 ± 9.37 mg/mL.

Previous studies on *A. bilimbi* have primarily focused on wound-healing applications using leaf extracts, while the fruit extract remains underutilized, particularly in topical anti-aging formulations. Creams are a preferred topical dosage form due to their ease of application, favorable aesthetics, and comfort on the skin.

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

Materials and Methods

Materials

Dried *Averrhoa 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).

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 dkk., 2019).

Cream Formulation

Creams containing *A. bilimbi* extract were prepared in concentrations of 1% (F1), 3% (F2), and 5% (F3). The formulation method referred to Iskandar et al. (Iskandar dkk., 2016). The composition of each cream formula is presented in **Table 1**. The water-soluble phase—comprising TEA, nipagin, and glycerin dissolved in distilled water—was heated to 70°C. Simultaneously, the oil phase—comprising stearic acid, cetyl alcohol, stearyl alcohol, and nipasol—was also heated to the same temperature. The aqueous phase was then gradually added to the oil phase under continuous stirring until a stable emulsion formed. Once the emulsion was uniform and slightly cooled, the extract was added and mixed thoroughly until homogeneous.

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

Ingredient	F1 (%)	F2 (%)	F3 (%)
A. bilimbi 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%.

Evaluation of Cream Characteristics

Organoleptic evaluation included visual observation of the cream's form, color, and odor. Homogeneity was assessed by spreading a small portion of the cream between two glass slides and checking for uniformity and absence of coarse particles. The pH was determined using a digital pH meter to ensure it remained within the optimal topical range of 4.5–6.5. Viscosity was measured using a Brookfield viscometer with R7 spindle and recorded in centipoise (cP) units (Ramdhan & Yusuf, 2023).

Ethical Clearance

Ethical approval for the study was granted by the Bioethics Commission for Medical and Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang, with approval number 313/V/2019/Bioethics Commission. The ethical clearance covered both animal testing and human trials, ensuring full compliance with ethical standards.

Irritation Test

The irritation test was conducted in accordance with OECD guidelines (OECD, 2021). On each rabbit, a 6 cm² area of shaved dorsal skin was treated with 0.5 g of cream and covered using a semi-occlusive bandage. Observations for erythema and edema were made at 1, 24, 48, and 72 hours, and results were scored using the Draize method (**Table 2**). The same procedure was applied to human subjects, with additional daily observation during the first three days of product 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

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.

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.

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.

Results and Discussion

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**.

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

Formula	Smell	Form	Color	Homogeneity
Base	*A. bilimbi* fruit	Semi solid	White	Homogeneous

F1	*A. bilimbi* fruit	Semi solid	Light yellow	Homogeneous
F2	*A. bilimbi* fruit	Semi solid	Deep Yellow	Homogeneous
F3	*A. bilimbi* fruit	Semi solid	Deep yellow	Homogeneous

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.

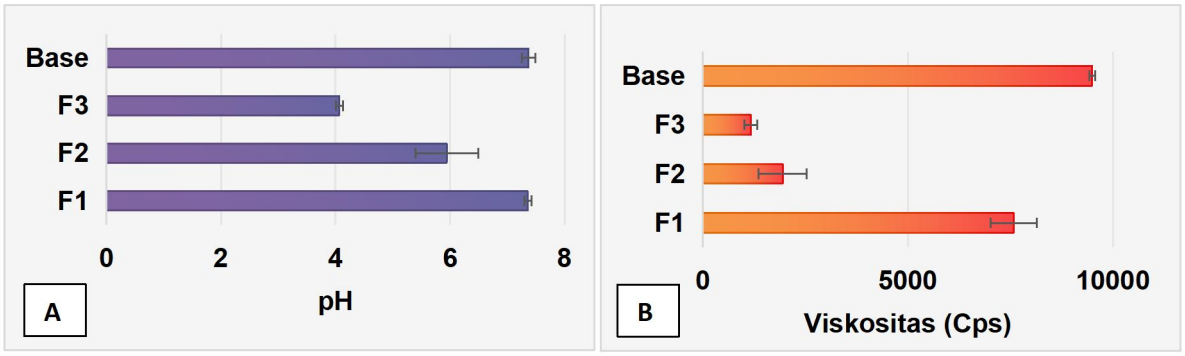

Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

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

Human Trial: Anti-Aging Parameters

Anti-aging efficacy was assessed by comparing skin parameters before and after 30 days of cream application. The skin condition improvements are visualized in Figure 2.

Sebum levels slightly increased in F2 and F3 groups, likely due to the higher oil content in W/O formulations. However, these changes were statistically non-significant ($p > 0.05$). Excessive sebum can obstruct pores, but the increase here did not exceed normal levels and may be counteracted by the formula's other active properties.

F3 showed the highest increase in skin hydration (22.34%) after 30 days of application. Nonetheless, this improvement was not statistically significant ($p > 0.05$). Moisturizing effects of flavonoid-rich extracts have been documented, but a longer duration may be needed to observe significant change (Rasul & Akhtar, 2012; Sugihartini, 2017).

F3 also exhibited the most effective pigmentation reduction (15.08%). However, statistical analysis did not support significance ($p > 0.05$). This result aligns with previous studies that reported visible depigmentation only after 8 weeks of application (Khan dkk., 2014).

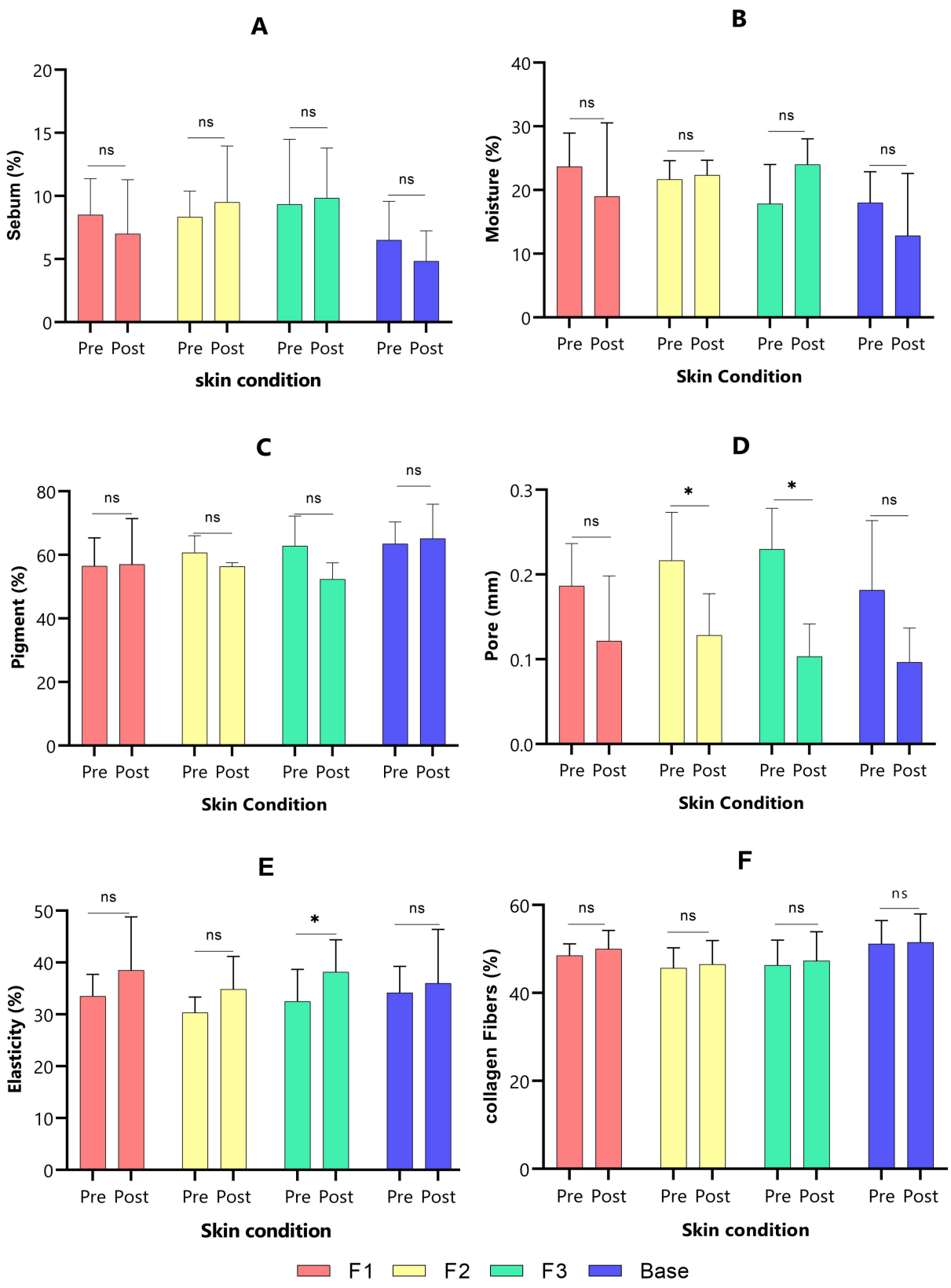

Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers, Mean ± SD (n=6), ns = no significant, * = significant (p<0,05) with t test

Significant reduction in pore diameter was observed in both F2 and F3 groups ($p < 0.05$), with F3 achieving the greatest reduction (54.18%). This effect may be attributed to phenolic compounds, including tannins, which act as astringents and help tighten skin pores (Puspitasari, 2011).

Skin elasticity improved significantly in the F3 group ($p < 0.05$), with an increase of 16.85%. This result suggests that *A. bilimbi* extract may contribute to collagen preservation or synthesis. Improved elasticity correlates well with the observed pore shrinkage, indicating restored dermal integrity (Jadoon dkk., 2015).

Collagen fiber density showed a minimal increase (1.12%) in the F3 group and was not statistically significant ($p > 0.05$). Collagen improvement may require prolonged use or higher extract concentrations.

Among the three cream formulations tested, F3 (5% *A. bilimbi* extract) demonstrated the most consistent improvements across multiple skin-aging parameters. Statistically significant changes were observed in pore size and elasticity, suggesting strong anti-aging potential. Although other parameters did not show statistical significance, trends in moisture and pigmentation improvement were evident. The results indicate that *A. bilimbi* extract is a promising active ingredient for anti-aging cosmetic formulations, particularly in W/O cream form.

Conclusion

Based on the findings from this study, the cream formulation containing 5% *Averrhoa 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 other parameters such as moisture content, pigmentation, and collagen fiber density showed positive trends, they did not reach statistical significance within the 30-day application period. The results support the use of *A. bilimbi* fruit extract in topical formulations for anti-aging purposes. Further studies with longer treatment durations and higher extract concentrations are recommended to strengthen and expand these findings.

Acknowledgment

The authors gratefully acknowledge the financial support provided by the Directorate of Research and Community Service (Direktorat Riset dan Pengabdian Masyarakat/DRPM), Ministry of Research, Technology, and Higher Education of the Republic of Indonesia, through the Inter-University Cooperation Program (Program Kerja Sama Perguruan Tinggi/PKPT). We also thank the volunteers and medical personnel at Rista Clinic Beauty Care, Demak, for their contribution to the clinical aspects of this study.

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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. MATERIALS AND METHODS

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 *Averrhoa bilimbi* Fruit Extract Cream Formulations

Ingredient	F1 (%)	F2 (%)	F3 (%)
A. bilimbi 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. Evaluation of Cream Characteristics

Organoleptic evaluation included observations of the cream's color, odor, and consistency. Homogeneity was assessed by spreading a sample between two glass slides to ensure uniformity and absence of coarse particles. The pH was measured with a digital pH meter to confirm suitability for topical use (pH 4.5–6.5). Viscosity was determined using a Brookfield viscometer with R7 spindle and recorded in centipoise (cP) (Ramdhan & Yusuf, 2023).

2.5. 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.

2.6. Irritation Test

Irritation tests were conducted in compliance with OECD guidelines (OECD, 2021). A 6 cm^2 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). he 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

2.7. 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.

2.8. 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.

2.9. 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.

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

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

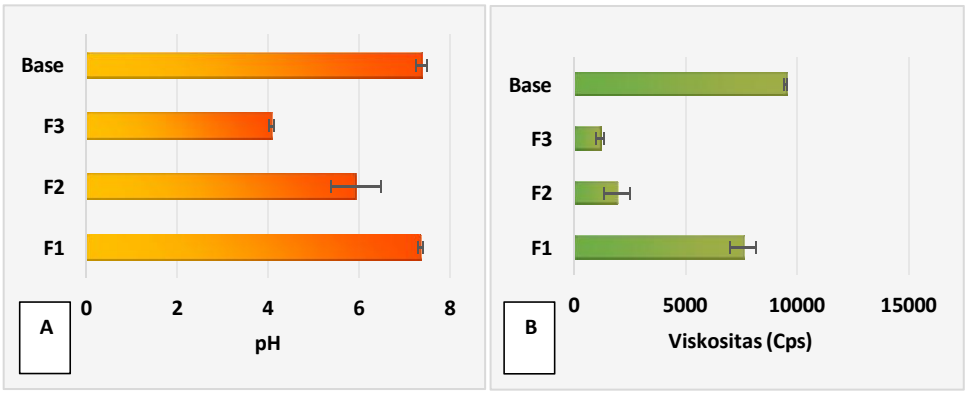

Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

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).

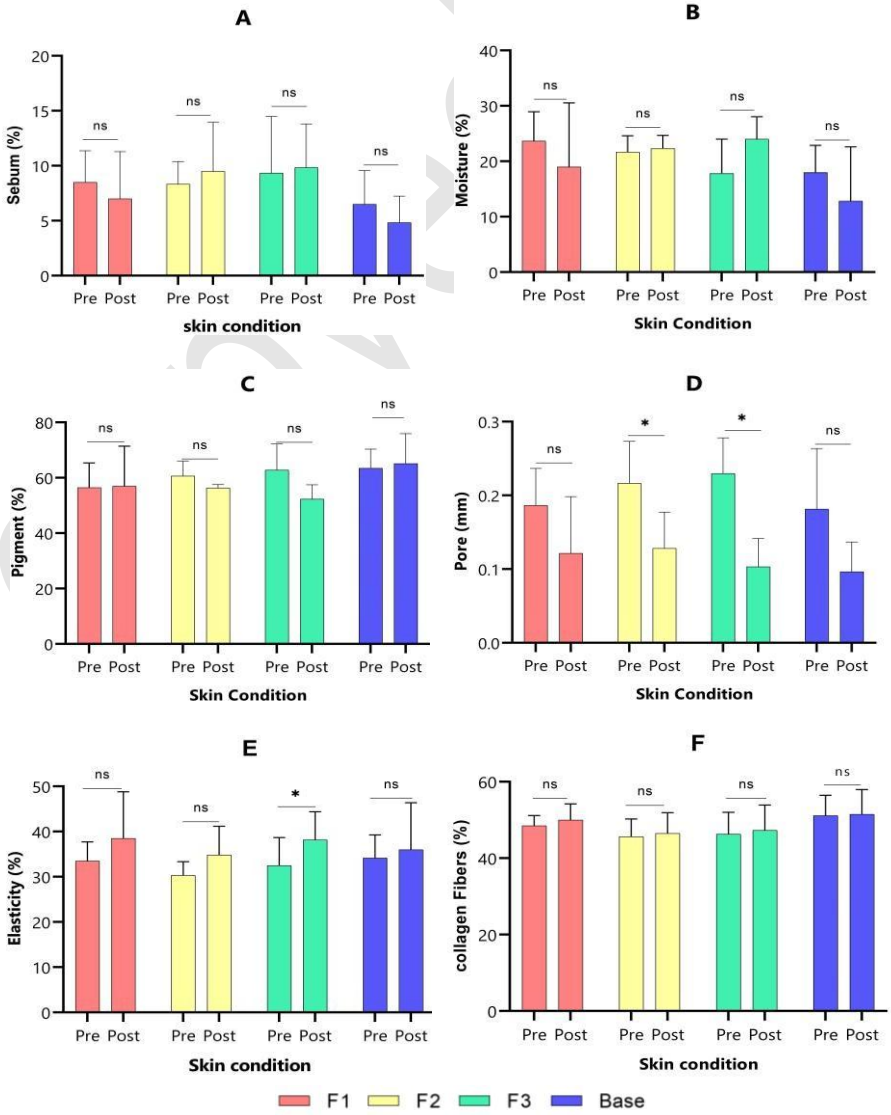

Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers, Mean ± SD (n=6), ns = no significant, * = significant ($p<0,05$) with t test.

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.

4. CONCLUSION

Based on the findings from this study, the cream formulation containing 5% *Averrhoa 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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Averrhoa bilimbi
Krim antipenuaan
Uji klinis
Elastisitas kulit
Pori-pori

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 (%)
A. bilimbi 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 cm^2 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**). he 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.

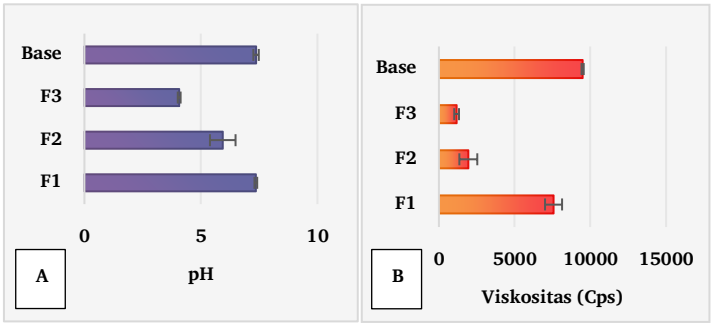

Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

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

Formula	Smell	Form	Color	Homogeneity
Base	*A. bilimbi fruit*	Semi solid	White	Homogeneous
F1	*A. bilimbi fruit*	Semi solid	Light yellow	Homogeneous
F2	*A. bilimbi fruit*	Semi solid	Deep Yellow	Homogeneous
F3	*A. bilimbi 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).

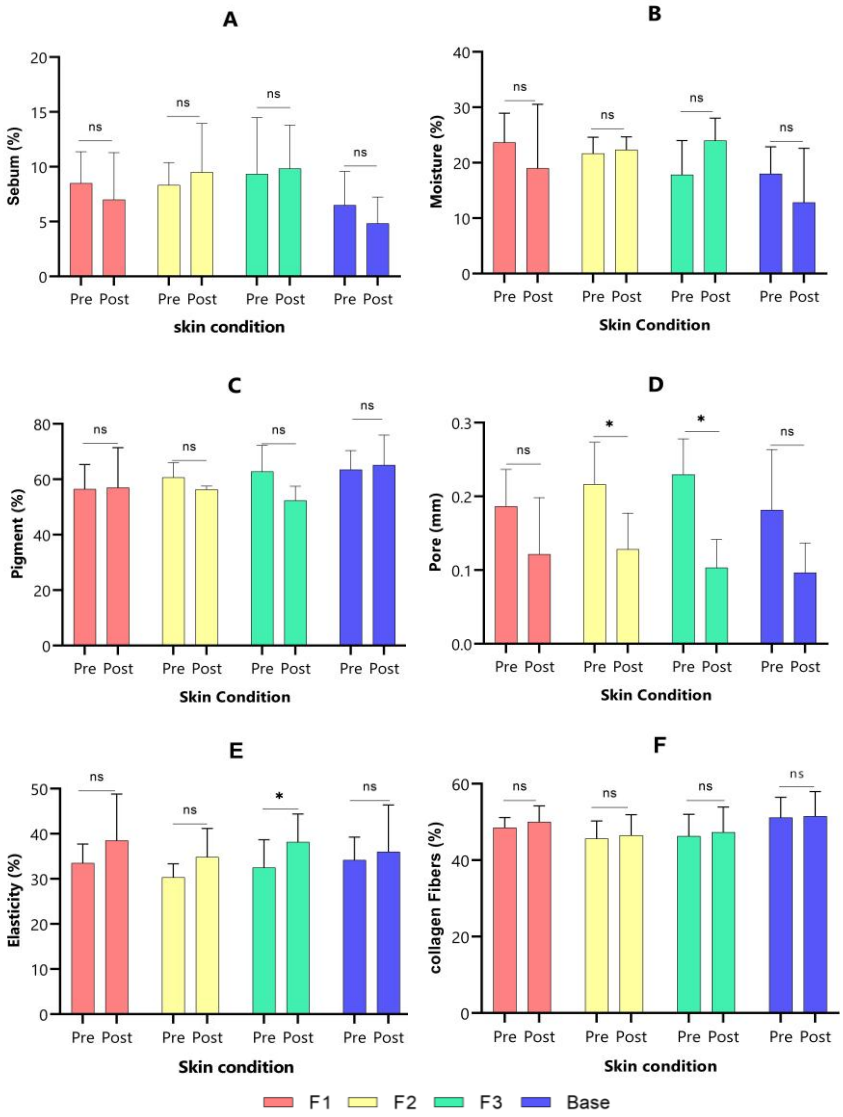

Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers, Mean ± SD (n = 6), ns = no significant, * = significant (p < 0,05) with t test

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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