

**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
1	Submission of manuscript	10 Mei 2025
2	Editorial Decision "Send to Review"	10 Mei 2025
3	Manuscript revision request	12 Mei 2025
4	Submir revised manuscript	12 Mei 2025
5	Acceptance Notification	13 Mei 2025
6	Payment Request	13 Mei 2025
7	Publication payment report	13 Mei 2025
8	Copyediting Revision Request "Revise manuscript based on the editor's comments"	13 Mei 2025
9	Manuscript Proof for Correction	15 Mei 2025
10	Editor Decision-Send to Production	26 Mei 2025
11	Article published	26 Mei 2025

# 1. Submission of Manuscript

← → ↻ jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401

**Jurnal Jamu Indonesia** 🔔 👤

← Back to Submissions

401 / **Suharsanti et al.** / Anti-Aging Efficacy of *Averrhoa bilimbi* Fruit Extract Cream: A Human Clinical Trial Library

Workflow **Publication**

Submission **Review** Copyediting Production

**Submission Files** 🔍 Search

▶	3409 for Review JJI-401 : Suharsanti et al. : Anti-aging.docx	May 10, 2025	Article Text
▶	3408 Ririn Suharsanti_JJI_10 Mei.docx	May 10, 2025	Article Text

[Download All Files](#)

**Pre-Review Discussions** ➕ Add discussion

**ririnsuharsanti@stifar.ac.id** 🔍 👤 🗑️ 🔄 🔄

Select Threads Options Empty Refresh

Reply Reply all Forward Delete Archive Not junk Mark More

**Inbox** 🔍 Search... 📧

- Thank you for your review  
ojs@journalnotific... 2025-05-13 17:16
- Copyediting Revision Request - "...  
ojs@journalnotific... 2025-05-13 16:52
- Payment Request  
ojs@journalnotific... 2025-05-13 16:48
- Article Acceptance Notification - ...  
ojs@journalnotific... 2025-05-12 14:12
- Thank you for your review  
ojs@journalnotific... 2025-05-12 06:27
- Article Review Request  
IJPRA Journal 2025-05-10 20:21
- DR. SANDI  
ojs@journalnotific... 2025-05-10 10:02
- Editor Decision  
ojs@journalnotific... 2025-05-10 09:16
- **Submission Acknowledgement**  
ojs@journalnotific... 2025-05-10 09:16
- Comments for the Editor

**Submission Acknowledgement** 🔔

From [ojs@journalnotification.com](mailto:ojs@journalnotification.com) on 2025-05-10 09:16  
[📧 Details](#) [📄 Headers](#) [📄 Plain text](#)

Ririn Suharsanti:

Thank you for submitting the manuscript, "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study" to Jurnal Jamu Indonesia. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: <https://jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401>  
Username: ririnsuharsanti

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

---

**Indonesian Journal of Jamu** (*Jurnal Jamu Indonesia*)  
<https://jamu-journal.ipb.ac.id/index.php/JJI/index>  
Bogor, INDONESIA

## 2. Editorial Decision

jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401

Jurnal Jamu Indonesia

← Back to Submissions

### Notifications

#### Editor Decision

2025-05-10 10:02 AM

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

We have reached a decision regarding your submission to {\$contextName}, "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study".

Our decision is to: Send to Review

Submission URL: <https://jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401>

Editor Decision	2025-05-10 10:02 AM
Revision Request	2025-05-10 09:47 AM
Article Acceptance Notification - "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human T...	2025-05-13 04:48 PM
Editor Decision	2025-05-26 11:40 AM

srv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?\_task=mail&\_mbox=INBOX.spam

ririnsuharsanti@stifar.ac.id

Select Threads Options Empty Refresh

Reply Reply all Forward Delete Archive Not junk Mark More

Inbox  
Drafts  
Sent  
Junk  
Trash  
Archive

Search...

- Thank you for your review  
ojs@journalnotific... 2025-05-13 17:16
- Copyediting Revision Request - "...  
ojs@journalnotific... 2025-05-13 16:52
- Payment Request  
ojs@journalnotific... 2025-05-13 16:48
- Article Acceptance Notification - ...  
ojs@journalnotific... 2025-05-12 14:12
- Thank you for your review  
ojs@journalnotific... 2025-05-12 06:27
- Article Review Request  
JJPRA Journal 2025-05-10 20:21
- DR. SANDI  
ojs@journalnotific... 2025-05-10 10:02
- Editor Decision  
ojs@journalnotific... 2025-05-10 09:16
- Submission Acknowledgement  
ojs@journalnotific... 2025-05-10 09:16
- Comments for the Editor

Messages 1 to 17 of 17

### Editor Decision

From ojs@journalnotification.com on 2025-05-10 10:02

Details Headers Plain text

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

We have reached a decision regarding your submission to {\$contextName}, "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study".

Our decision is to: Send to Review

Submission URL: <https://jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401>

### 3. Manuscript revision request

The screenshot shows an email interface with the sender 'ojs@journalnotification.com' and the subject 'Revision Request for Manuscript ID JJI-401 – Jurnal Jamu Indonesia'. The email body contains the following text:

Dear Ririn Suharsanti, Muhammad Ryan Radix Rahadhian, Nining Sugihartini, Endang Lukitaningsih,

We have reached a decision regarding your submission to *Jurnal Jamu Indonesia*, entitled "**Anti-aging Potential of *Averrhoa bilimbi* Fruits Cream: a Human Trial Study.**"

**Our decision is: Revisions Required**

Please revise your manuscript according to the *Jurnal Jamu Indonesia* guidelines, which can be accessed here: <https://drive.google.com/file/d/1qHEPhEkhwi2mmaOA3FP4nUUZVygiMwQY/view>, and incorporate the suggestions and recommendations provided by the reviewers. The revised manuscript must be submitted within **2 days**.

In addition to the reviewer suggestions, please ensure the following:

- **Reduce the similarity index** of the manuscript to **below 25%**, as currently it stands at **55%** (see attached file).
- **Include the full names of all authors, their respective affiliations, and complete corresponding author information** on the manuscript's title page.
- **Highlight all revised sections in yellow** within the manuscript.

The screenshot shows a web browser window with the URL 'jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401'. A notification pop-up is displayed over the page, containing the same revision request text as the email above. The notification includes the subject 'Revision Request for Manuscript ID JJI-401 – Jurnal Jamu Indonesia', the date '2025-05-12 05:47 AM', and the same body text regarding the decision to require revisions and the list of requirements.

## 4. Progress revision results

jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401

Jurnal Jamu Indonesia

← Back to Submissions

### Revisions

Search Upload File

3431	Turnitin_Ririn Suharsanti_JJI_12 Mei 2025.pdf	May 12, 2025	Other
3430	JJI_Author's Response Letter.pdf	May 12, 2025	Other
3429	Ririn Suharsanti_JJI_12 Mei.pdf	May 12, 2025	Article Text
3427	Ririn Suharsanti_JJI_12 Mei.docx	May 12, 2025	Article Text

### Review Discussions

Add discussion

Name	From	Last Reply	Replies	Closed
<a href="#">Confirm of Revision</a>	ririnsuharsanti	-	0	<input type="checkbox"/>

jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401

Jurnal Jamu Indonesia

← Back to Submissions

### Messages

Note	From
<p>Dear editor,</p> <p>We have revised our manuscript, all corrections have been highlighted in <b>yellow</b> and we have written them in detail in the <b>Author's Response Letter file</b>.</p> <p>Hopefully it will be well received and the manuscript will be worthy for publish</p> <p><b>we have deleted the turnitin repository of the institution</b> 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 (9%).</p> <p>Regard,</p> <p>Ririn Suharsanti</p> <p><a href="#">Turnitin_Ririn Suharsanti_JJI_12 Mei 2025.pdf</a></p> <p><a href="#">JJi_Author's Response Letter.pdf</a> <a href="#">Ririn Suharsanti_JJI_12 Mei.pdf</a></p> <p><a href="#">Ririn Suharsanti_JJI_12 Mei.docx</a></p>	ririnsuharsanti 2025-05-12 06:29 PM

Add Message

## 5. Acceptance Notification

→ [rv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?\\_task=mail&\\_mbox=INBOX.spam](mailto:rv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?_task=mail&_mbox=INBOX.spam)

ririnsuharsanti@stifar.ac.id

Select Threads Options Empty Refresh

Inbox Article Acceptance Notifical x 2  
ojs@journalnotificatio... 2025-05-13 16:48  
• Article Acceptance Notification – "A..."

Drafts

Sent

Junk

Trash

Archive

Reply Reply all Forward Delete Archive Not junk Mark More

### Article Acceptance Notification – "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study"

From ojs@journalnotification.com on 2025-05-13 16:48  
[Details](#) [Headers](#) [Plain text](#)

Dear Ririn Suharsanti,

We are pleased to inform you that your manuscript entitled "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study" has been **accepted for publication** in **Jurnal Jamu Indonesia**, following a thorough peer review and editorial assessment.

Your article has met the required standards for scientific quality, originality, and relevance to the journal's scope. It will proceed to the **production stage (layout and typesetting) after the required publication fee has been successfully paid**.

Please make the payment as per the instructions provided on our journal's website or the payment invoice. Once your payment is confirmed, your article will enter the final processing stage.

If you have any questions regarding payment or the next steps, please do not hesitate to contact us. Thank you for choosing Jurnal Jamu Indonesia as the venue for your research.

Regards,  
**Prof. Dr. Waras Nurcholis, SSI, MSI**  
Editor-in-Chief  
[Jurnal Jamu Indonesia \(Indonesian Journal of Jamu\)](#)

← → ↻ [jamu-journal.iipb.ac.id/index.php/IJI/authorDashboard/submission/401](http://jamu-journal.iipb.ac.id/index.php/IJI/authorDashboard/submission/401)

Jurnal Jamu Indonesia

← Back to Submissions

### Article Acceptance Notification – "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study"

2025-05-13 04:48 PM

Dear Ririn Suharsanti,

We are pleased to inform you that your manuscript entitled "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study" has been **accepted for publication** in **Jurnal Jamu Indonesia**, following a thorough peer review and editorial assessment.

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Regards,  
**Prof. Dr. Waras Nurcholis, SSI, MSI**  
Editor-in-Chief  
[Jurnal Jamu Indonesia \(Indonesian Journal of Jamu\)](#)

0:02 AM

5:47 AM

4:48 PM

1:40 AM

Search

## 6. Payment Request

The screenshot shows an email client interface. The address bar displays the URL: `srv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?_task=mail&_mbox=INBOX.spam`. The email header shows the sender as `ojs@journalnotification.com` on 2025-05-13 16:52. The subject is "Payment Request". The email body contains the following text:

Dear Authors,

Congratulations, your article has been accepted for publication in **Jurnal Jamu Indonesia**.

The publication fee is **IDR 2,000,000 (fast track) and IDR 500,000 (improve English) (Total IDR 2,500,000)**. Please transfer the payment to:

Bank Name: Bank Mandiri  
Account Holder: WARAS NURCHOLIS  
Account Number: 1330025002254

The payment deadline is **five calendar days** from the date of this email.

Once payment is made, please confirm by uploading the proof of payment in the **OJS Jurnal Jamu Indonesia System** using the **Add comments** tab, and also send it via email to [wnurcholis@apps.ipb.ac.id](mailto:wnurcholis@apps.ipb.ac.id) and [jurnaljamuindonesia@apps.ipb.ac.id](mailto:jurnaljamuindonesia@apps.ipb.ac.id).

We are pleased to inform you that **Jurnal Jamu Indonesia** is currently accredited **SINTA 2** (see [SINTA profile](#)) and is under evaluation for indexing in **Scopus** (see [progress here](#)).

Your quick response will help us to publish your article in the upcoming issue.

Thank you for your valuable contribution.

Regards,  
**Waras Nurcholis**

The screenshot shows the author dashboard for Jurnal Jamu Indonesia. The URL is `jamu-journal.ipb.ac.id/index.php/JII/authorDashboard/submission/401`. A modal window titled "Payment Request" is open, displaying the following information:

**Participants**

- Waras Nurcholis (wnurcholis)
- Ririn Suharsanti (ririnsuharsanti)

**Messages**

Note	From
Dear Authors,	wnurcholis
Congratulations, your article has been accepted for publication in <b>Jurnal Jamu Indonesia</b> .	2025-05-13 04:50 PM
The publication fee is <b>IDR 2,000,000 (fast track) and IDR 500,000 (improve English) (Total IDR 2,500,000)</b> . Please transfer the payment to:	
Bank Name: Bank Mandiri Account Holder: WARAS NURCHOLIS Account Number: 1330025002254	
The payment deadline is <b>five calendar days</b> from the date of this email.	
Once payment is made, please confirm by uploading the proof of payment in the <b>OJS Jurnal Jamu Indonesia System</b> using the <b>Add comments</b> tab, and also send it via	

## 7. Publication payment report

srv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?\_task=mail&\_mbox=INBOX.Sent

ririnsuharsanti@stifar.ac.id

Select Threads Options Refresh

Reply Reply all Forward Delete Archive Junk Mark More

Inbox

Drafts

Sent

Junk

Trash

Archive

Search: jji

wnurcholis@apps.ipb... 2025-05-13 17:10

- PAYMENT JJI-401-RIRIN SUHARSA...

### PAYMENT JJI-401-RIRIN SUHARSANTI

To wnurcholis@apps.ipb.ac.id, 1 more... on 2025-05-13 17:10

Details Headers

BUKTI BAYAR JJI RIRIN SUHARSANTI.jpeg (~47 KB)

Editor yg terhormat,

Terlampir bukti bayar untuk artikel dengan ID JJI-401

Semoga dapat diterima dengan baik

terimakasih  
Ririn Suharsanti

BUKTI BAYAR JJI RIRIN SUHARSANTI.jpeg ~47 KB

Messages 1 to 1 of 1

jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401#

### Jurnal Jamu Indonesia

← Back to Submissions

401 / Suharsanti

Workflow

Submissions

Copy

Name

Payment

Once payment is made, please confirm by uploading the proof of payment in the OJS Jurnal Jamu Indonesia System using the **Add comments** tab, and also send it via email to [wnurcholis@apps.ipb.ac.id](mailto:wnurcholis@apps.ipb.ac.id) and [jurnaljamuindonesia@apps.ipb.ac.id](mailto:jurnaljamuindonesia@apps.ipb.ac.id).

We are pleased to inform you that **Jurnal Jamu Indonesia is currently accredited SINTA 2** (see [SINTA profile](#)) and is **under evaluation for indexing in Scopus** (see [progress here](#)).

Your quick response will help us to publish your article in the upcoming issue.

Thank you for your valuable contribution.

Regards,  
**Waras Nurcholis**  
Editor-in-Chief  
*Jurnal Jamu Indonesia*

▶ Kami kirimkan bukti bayar melalui OJS

BUKTI BAYAR JJI RIRIN SUHARSANTI.jpeg

ririnsuharsanti  
2025-05-14 09:17 PM

Message \*

📄 📧 B I U 🔗 🌐 ⏪ ⏩ 🖼️ 📎

Library

Discussion

Closed

## 8. Copyediting Revision Request

The screenshot shows an email interface for the address `ririnsuharsanti@stifar.ac.id`. The email subject is "Copyediting Revision Request - 'Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study'". It is from `ojs@journalnotification.com` dated 2025-05-13 17:16. An attachment is visible: "401 : Suharsanti et al. : Editor improved.docx (~478 KB)".

Dear Authors,

Thank you for your continued work on the manuscript entitled "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study".

As part of the copyediting process, the editorial team has reviewed your manuscript and found that some revisions are needed before we can proceed to the final production stage.

Please:

- Revise your manuscript based on the editor's comments.
- Highlight all changes made in yellow.
- Upload the revised version directly in the Copyediting stage.

We kindly ask that you submit your revision within 2 days of this email. If you require more time, please notify us as soon as possible.

Thank you for your cooperation.

Best regards,  
**Prof. Dr. Waras Nurcholis, SSI, MSI**  
Editor-in-Chief, Jurnal Jamu Indonesia

The screenshot shows a web dashboard for "Jurnal Jamu Indonesia" with a modal window titled "Copyediting Revision Request - 'Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study'".

**Participants**

- Waras Nurcholis (wnurcholis)
- Ririn Suharsanti (ririnsuharsanti)

**Messages**

Note	From
Dear Authors,	wnurcholis
Thank you for your continued work on the manuscript entitled "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study".	2025-05-13 05:15 PM
As part of the copyediting process, the editorial team has reviewed your manuscript and found that some revisions are needed before we can proceed to the final production stage.	
Please:	
- Revise your manuscript based on the editor's comments.	
- Highlight all changes made in yellow.	
- Upload the revised version directly in the Copyediting stage.	

## 9. Manuscript Proof for Correction

jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401#

Jurnal Jamu Indonesia

← Back to Submissions

### Manuscript Proof for Correction

**Participants**

- Waras Nurcholis (wnurcholis)
- Ririn Suharsanti (ririnsuharsanti)
- Novian Liwanda (nliwanda)

**Messages**

Note	From
Dear Ririn Suharsanti,	nliwanda
We hope this message finds you well.	2025-05-15 12:17 PM
Please find attached the proof of your manuscript. We kindly request that you carefully check the content for errors or corrections. All changes should be clearly highlighted using yellow color or track changes.	
Once completed, please return the corrected version at your earliest convenience.	
If you have any questions or require further assistance, please do not hesitate to contact us.	

srv135.niagahoster.com:2096/cpsess3287728682/3rdparty/roundcube/?\_task=mail&\_mbox=INBOX.spam

ririnsuharsanti@stifara.ac.id

Select Threads Options Empty Refresh

Reply Reply all Forward Delete Archive Not junk Mark More

**Manuscript Proof for Correction**

From ojs@journalnotification.com on 2025-05-15 12:24

401+Proof+Read+Suharsanti+et+al.+2025.pdf (~494 KB)

Dear Ririn Suharsanti,

We hope this message finds you well.

Please find attached the proof of your manuscript. We kindly request that you carefully check the content for errors or corrections. All changes should be clearly highlighted using yellow color or track changes.

Once completed, please return the corrected version at your earliest convenience.

If you have any questions or require further assistance, please do not hesitate to contact us.

Thank you for your cooperation.

Best regards,  
Editor

Messages 1 to 17 of 17

# 10. Editor Decision-Send to Production

The screenshot shows an email client interface for the account `ririnsuharsanti@stifar.ac.id`. The left sidebar lists folders: Inbox, Drafts, Sent, Junk, Trash, and Archive. The main pane displays a list of messages, with the selected message titled "Editor Decision" from `ojs@journalnotification.com` dated 2025-05-26 11:40. The email content includes:

**Editor Decision**

From `ojs@journalnotification.com` on 2025-05-26 11:40

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

The editing of your submission, "Anti-Aging Efficacy of Averrhoa bilimbi Fruit Extract Cream: A Human Clinical Tria," is complete. We are now sending it to production.

Submission URL: <https://jamu-journal.ipb.ac.id/index.php/JJI/authorDashboard/submission/401>

The screenshot shows the author dashboard for "Jurnal Jamu Indonesia". The page title is "Jurnal Jamu Indonesia" and the user is logged in as "ririnsuharsanti". A "Back to Submissions" link is visible. The main content area displays a table of submission messages:

Name	From	Last Reply	Replies	Closed
<a href="#">Payment Request</a>	wnurcholis 2025-05-13 04:50 PM	ririnsuharsanti 2025-05-14 09:17 PM	1	<input type="checkbox"/>
<a href="#">Copyediting Revision Request - "Anti-aging Potential of Averrhoa bilimbi Fruits Cream : a Human Trial Study"</a>	wnurcholis 2025-05-13 05:15 PM	ririnsuharsanti 2025-05-14 09:18 PM	1	<input type="checkbox"/>
<a href="#">Manuscript Proof for Correction</a>	nliwanda 2025-05-15 12:17 PM	ririnsuharsanti 2025-05-15 01:00 PM	1	<input type="checkbox"/>

Below the table, there is a "Copyedited" section with a search bar and a list of items:

Copyedited	Search
3527 10(2)9 401 - Draft Research Article.pdf	May 26, 2025 Article Text





44 viskositas. Uji iritasi dilakukan pada hewan. Semua krim juga dievaluasi efek  
45 anti-penuaannya dengan parameter sebum, kelembaban, pigmen, pori-pori,  
46 elastisitas, dan serat kolagen dengan skin analyzer pada subjek manusia yang  
47 telah menggunakan krim selama 30 hari. Hasil penelitian menunjukkan bahwa  
48 F3 (5%) memiliki efek anti-penuaan terbaik dengan meningkatkan kelembaban,  
49 mengurangi pigmentasi, mengecilkan pori-pori, dan meningkatkan elastisitas  
50 kulit. Setelah pengujian statistik t test pada masing-masing parameter anti-  
51 aging, krim F3 telah terbukti efektif memiliki potensi untuk memperkecil pori-  
52 pori kulit dan meningkatkan elastisitas kulit.

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

### 55 Introduction

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

69 Cosmetic products have one of the benefits to protect the skin because of  
70 the ingredients they contain, so they can affect the biological function of the  
71 skin. Some plant extracts and antioxidants obtained from natural sources are  
72 able to prevent aging and can improve skin health. To prevent skin from aging  
73 or wrinkles, natural phytochemical sources are preferred. Plant extracts rich in  
74 secondary metabolites, such as flavonoids, phenolic acids, saponins and  
75 alkaloids, which have collagen synthesis activity, are being widely used for the  
76 development of topical anti-aging skin cosmetic products (Suharsanti et al.,  
77 2019). One of the natural sources that contains phenolic compounds, flavonoids  
78 is *Averrhoa bilimbi* fruit.

79 The total phenolic content of *Averrhoa bilimbi* fruits extract is  $19.8022 \pm$   
80  $1.6696$  mg GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g  
81 and total flavonoids  $24.7458 \pm 0.3291$  mg RE/g more than leaves ethanolic  
82 extract  $13.3126 \pm 0.5695$  mg RE/g. The SPF (Sun Protecting Factor) value of the  
83 ethanol extract concentration of 300 ppm fruit can be described in the category  
84 of sunblock assessment with maximum protection. *Averrhoa bilimbi* fruit  
85 extract has an  $IC_{50}$  value of antioxidant activity of  $2.33 \pm 0.33$  mg/ml and  
86 tyrosinase inhibitor activity with an  $IC_{50}$  value of  $186.85 \pm 9.37$  mg/mL  
87 (Suharsanti et al., 2019). Research on *Averrhoa bilimbi* has been conducted for  
88 wound healing but on leaf samples. Based on the results of the study  
89 qualitatively ethanol extract 70% *Averrhoa bilimbi* fruits contains flavonoids,  
90 alkaloids, saponins, and terpenoids and quantitatively has an  $IC_{50}$  value of  
91 74,625 ppm which is included in the category of strong antioxidants (Kusuma  
92 et al., 2023). In other research, The test results of antioxidant activity with the  
93 DPPH method using the most effective UV-Vis spectrophotometer as the  
94 antioxidant cream of the *Averrhoa bilimbi* leaves extract has a value of  $IC_{50}$   
95 0.118 ppm and vitamin C as comparator has a value of  $IC_{50}$  0.0327 ppm (Ariem  
96 et al., 2020). Shooting gel preparation with a combination of *Aloe vera* and  
97 *Averrhoa bilimbi* fruits formula I (85%) can increase the moisture value of  
98 human skin between before and after use (Ariani & Suharsanti, 2018a). Cream  
99 containing of 2% Bilimbi leaf extract (*Averrhoa bilimbi*) has favorable physical  
100 characteristics and effectively heals incision wounds in male white mice (Suena  
101 et al., 2024). The irritation test showed that all concentrations of the shooting  
102 gel preparation formula didn't cause irritation to the skin of mice (Ariani &  
103 Suharsanti, 2018b). Based on this, *Averrhoa bilimbi* fruit which has been proven  
104 to contain efficacious compounds and has activity as a skin whitener in vitro  
105 needs to be developed into an anti-aging skin cosmetic preparation.

## 106 Methods

### 107 a. Material

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

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

114 **b. Extraction of *Averrhoa bilimbi***

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

118 **c. Formula Preparation**

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

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

129 **d. Physical Characteristics of Anti-aging Cream**

130 ***Organoleptic Test***

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

134 ***Homogeneity Test***

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

140 ***pH Test***

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

### 145 **Viscosity Test**

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

### 150 **e. Ethical Clearance**

151 Ethical clearance is a written ethical eligibility issued by the  
152 Medical/Health Research Bioethics Commission of the Faculty of Medicine,  
153 Sultan Agung Islamic University, Semarang with the number No.  
154 313/V/2019/Bioethics Commission after meeting the established requirements.  
155 The creation of ethical clearance is necessary because this study uses living  
156 subjects, test animals and humans, so it is necessary to ensure that this study  
157 has met the principle of respecting human dignity.

### 158 **f. Irritation Test on test animals**

159 Irritation Test was conducted with the help of New Zealand Rabbits aged  
160 12-16 weeks with the OECD method. The fur of the back and sides was shaved  
161 carefully  $\pm$  24 hours before application. The area is about 6 cm<sup>2</sup>. Apply 0.5 g of  
162 cream to each formula. Wrap the area with a semi-occlusive bandage (half-  
163 closed, but still allows air exchange). Observe the animal after 1 hour, 24 hours,  
164 48 hours, and 72 hours directly to the open skin. Irritation tests were also  
165 conducted on humans with the same treatment. Observe the presence of  
166 erythema (redness) and edema (swelling) and write down the irritation score  
167 (Draize Scoring System) according to table 2. Calculate the average score for  
168 each parameter at 24, 48, and 72 hours (OECD, 2021). Irritation conditions  
169 were also observed during application of the cream to humans during the initial  
170 3 days of use.

### 171 **g. Informed Consent**

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

### 183 **h. Human Trial of Anti-aging Cream**

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

### 195 **i. Analysis Data**

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

## 199 **Results and Discussion**

### 200 **a. Characteristics of Anti-aging Cream**

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

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

205 The results of the pH test on anti-aging creams F1, F2, and F3 can be seen  
206 in Figure 1A. The increase in pH value occurs as the extract increases but is still  
207 within a safe pH range. The requirements for a good pH preparation are 4.5 -  
208 8.0 to match the natural pH of the skin and prevent irritation (Ariem et al.,  
209 2020; SNI, 1996). Viscosity also increases with increasing extract but remains  
210 within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality  
211 requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI,  
212 1996; Firdaus et al., 2024). So all cream preparation formulas have met the pH  
213 and viscosity quality requirements. The viscosity results for F1, F2, and F3 can  
214 be seen in Figure 1B.

#### 215 **b. Irritation Test of Anti-aging Cream**

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

#### 222 **c. Human Trial of Anti-aging Cream**

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

#### 230 **Skin Sebum**

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

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

240 The skin sebum condition was reduced well after using F1 anti-aging  
241 cream compared to F2 and F3. The increase in sebum levels after the use of  
242 bases and formulations in F2 and F3 may be due to the oily nature of the W/O  
243 emulsion which has a thick oily liquid of stearic acid and glycerin (Rowe et al.,  
244 2006). Increased sebum also occurs in the topical cream of *Calendula officinalis*  
245 Extract formula due to the W/O emulsion type uses liquid paraffin (Akhtar et  
246 al., 2011). The results of the statistical test with paired t test on each  
247 concentration (base, F1, F2, F3) with baseline (skin condition before being  
248 given anti-aging cream) didn't show any effect of using anti-aging cream on  
249 skin sebum condition ( $\text{sig} > 0.05$ ).

#### 250 **Skin Moisture**

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

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

265 increased skin moisture by 22,34%. Although F3 has been able to increase skin  
266 moisture, statistics show something different. The results of statistical tests  
267 with paired t-tests on each concentration with baseline (skin condition before  
268 being given anti-aging cream) did not show any effect of using anti-aging  
269 cream on skin moisture because  $\text{sig} > 0.05$ . Several studies on cream formulas  
270 from extracts have also been able to increase skin moisture, such as in anti-  
271 aging potential of a cream containing milk thistle extract. The increase in skin  
272 moisture after application of the formulation may be due to the flavonoids from  
273 milk thistle because flavonoids increase the water content in the skin due to  
274 swelling of corneocytes on the skin surface (Rasul & Akhtar, 2012). Due to the  
275 presence of vitamin E and essential fatty acids, *Sauropus Androgynus* leaf  
276 extract may help moisture retention in the epidermis and resistance to dryness  
277 (Siallagan et al., 2024). *Averrhoa bilimbi* fruit also contains phenolic and  
278 flavonoid content which may also have an effect on increasing skin moisture  
279 activity (Rahardhian et al., 2020). With the paired sample t-test it was clear  
280 that a significant difference in skin moisture values was produced at week  
281 2 when the base was compared with the formulation (Akhtar et al., 2011).

### 282 **Skin Pigment**

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

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

#### 304 **Skin Pores**

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

315 F3 anti-aging cream is able to reduce the diameter of skin pores better  
316 than F1 and F2. Based on figure 2, F3 has the ability to shrink pores by up to  
317 54.18%. The results of statistical tests with paired t-tests on each concentration  
318 with baseline (skin condition before being given anti-aging cream) show that  
319 anti-aging F2 and F3 cream has an effect on the condition of skin pores because  
320  $\text{sig} < 0.05$ . It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have  
321 anti-aging activity in shrinking pores.

#### 322 **Skin Elasticity**

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

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

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

### 339 ***Skin Collagen Fibers***

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

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

### 352 **Conclusion**

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

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

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- 469

470 **Table 1.** *Averrhoa bilimbi* 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

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

Formula	Organoleptic			Homogeneity
	Smell	Form	Colour	
<b>Base</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	White	Homogeneous
<b>F1</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
<b>F2</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
<b>F3</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

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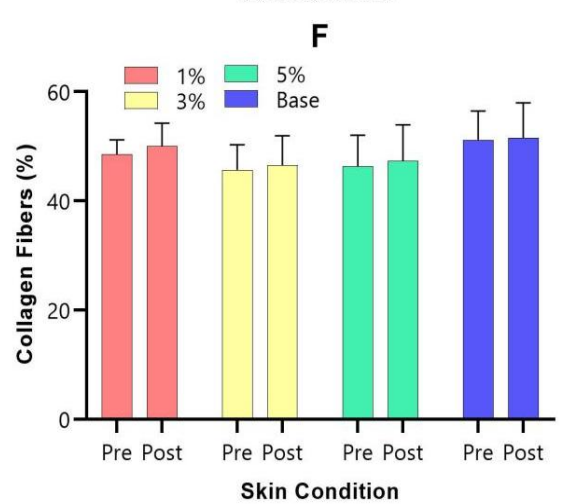
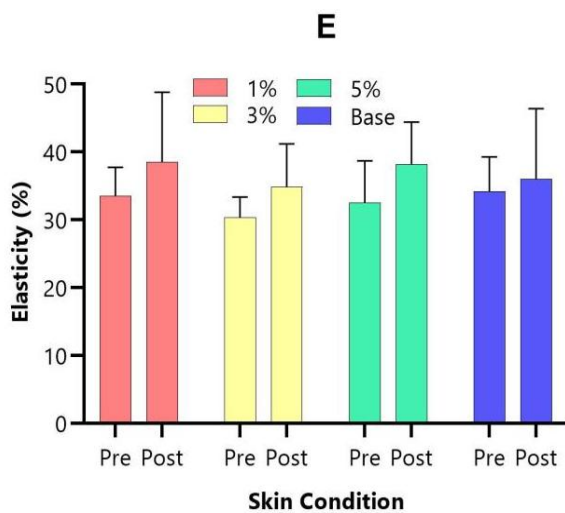
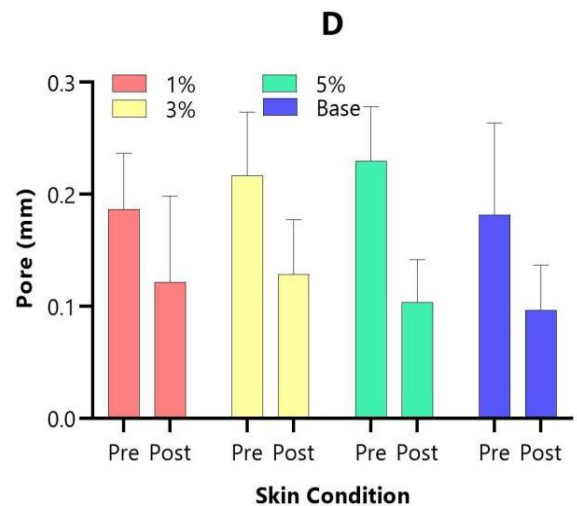
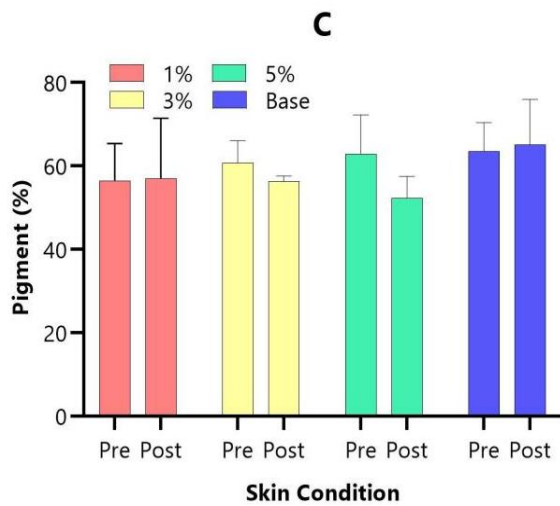
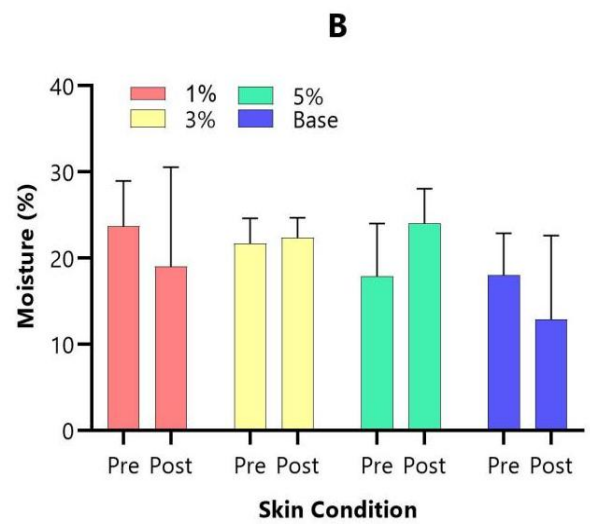
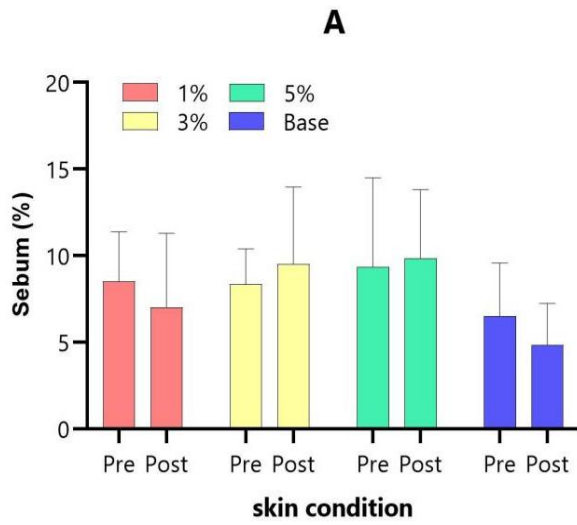
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**Figure 1.** Results of pH (A) and Viscosity (B) tests on anti-aging cream

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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

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

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10   **Abstract**

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

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

28   **Abstrak**

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

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

46 **Introduction**

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

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

67 The total phenolic content of *Averrhoa bilimbi* fruits extract is  $19.8022 \pm 1.6696$  mg  
68 GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g and total flavonoids  
69  $24.7458 \pm 0.3291$  mg RE/g more than leaves ethanolic extract  $13.3126 \pm 0.5695$  mg RE/g. The  
70 SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can  
71 be described in the category of sunblock assessment with maximum protection. *Averrhoa*  
72 *bilimbi* fruit extract has an  $IC_{50}$  value of antioxidant activity of  $2.33 \pm 0.33$  mg/ml and  
73 tyrosinase inhibitor activity with an  $IC_{50}$  value of  $186.85 \pm 9.37$  mg/mL (Suharsanti et al.,  
74 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf  
75 samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa*  
76 *bilimbi* fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has

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

### Methods

#### a. Material

93 *Averrhoa bilimbi* dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin,  
94 stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor,  
95 Indonesia), New Zealand Rabbits aged 12-16 weeks, Volunteers include subjects aged 19–  
96 25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State),  
97 skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

#### b. Extraction of *Averrhoa bilimbi*

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

#### c. Formula Preparation

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

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

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

### 111 **d. Physical Characteristics of Anti-aging Cream**

#### 112 ***Organoleptic Test***

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

#### 115 ***Homogeneity Test***

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

#### 120 ***pH Test***

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

#### 124 ***Viscosity Test***

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

### 128 **e. Ethical Clearance**

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

### 135 **f. Irritation Test on test animals**

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

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

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141 tests were also conducted on humans with the same treatment. Observe the presence of  
142 erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring  
143 System) according to table 2. Calculate the average score for each parameter at 24, 48, and  
144 72 hours (OECD, 2021). Irritation conditions were also observed during application of the  
145 cream to humans during the initial 3 days of use.

### 146 **g. Informed Consent**

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

### 157 **h. Human Trial of Anti-aging Cream**

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

### 168 **i. Analysis Data**

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

172 **Results and Discussion**

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### 173 a. Characteristics of Anti-aging Cream

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

178 The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure  
179 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range.  
180 The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin  
181 and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing  
182 extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality  
183 requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus  
184 et al., 2024). So all cream preparation formulas have met the pH and viscosity quality  
185 requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

### 186 b. Irritation Test of Anti-aging Cream

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

### 192 c. Human Trial of Anti-aging Cream

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

### 199 *Skin Sebum*

200 Sebum is part of the oil found on the surface of the skin. Sebum is produced by the  
201 sebaceous glands, or oil glands, and plays a role in helping to keep the skin and hair moist  
202 (Jadoon et al., 2015). Its components consist of triglycerides, free fatty acids, wax esters,  
203 squalene, cholesterol esters, and cholesterol. Too much sebum can cause body odor. Sebum  
204 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

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

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

### 217 **Skin Moisture**

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

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

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

### 245 **Skin Pigment**

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

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

### 264 **Skin Pores**

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

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

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

### 279 **Skin Elasticity**

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

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

### 294 **Skin Collagen Fibers**

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

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

### Conclusion

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

### Acknowledgment

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**Table 1.** *Averrhoa bilimbi* 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

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

Formula	Organoleptic			Homogeneity
	Smell	Form	Colour	
<b>Base</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	White	Homogeneous
<b>F1</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
<b>F2</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
<b>F3</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

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**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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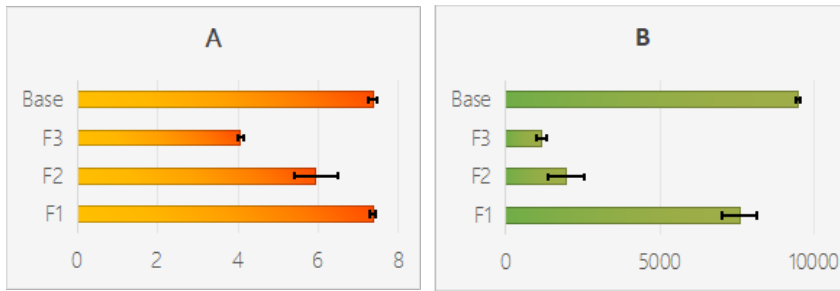


Figure 1. Results of pH (A) and Viscosity (B) tests on anti-aging cream

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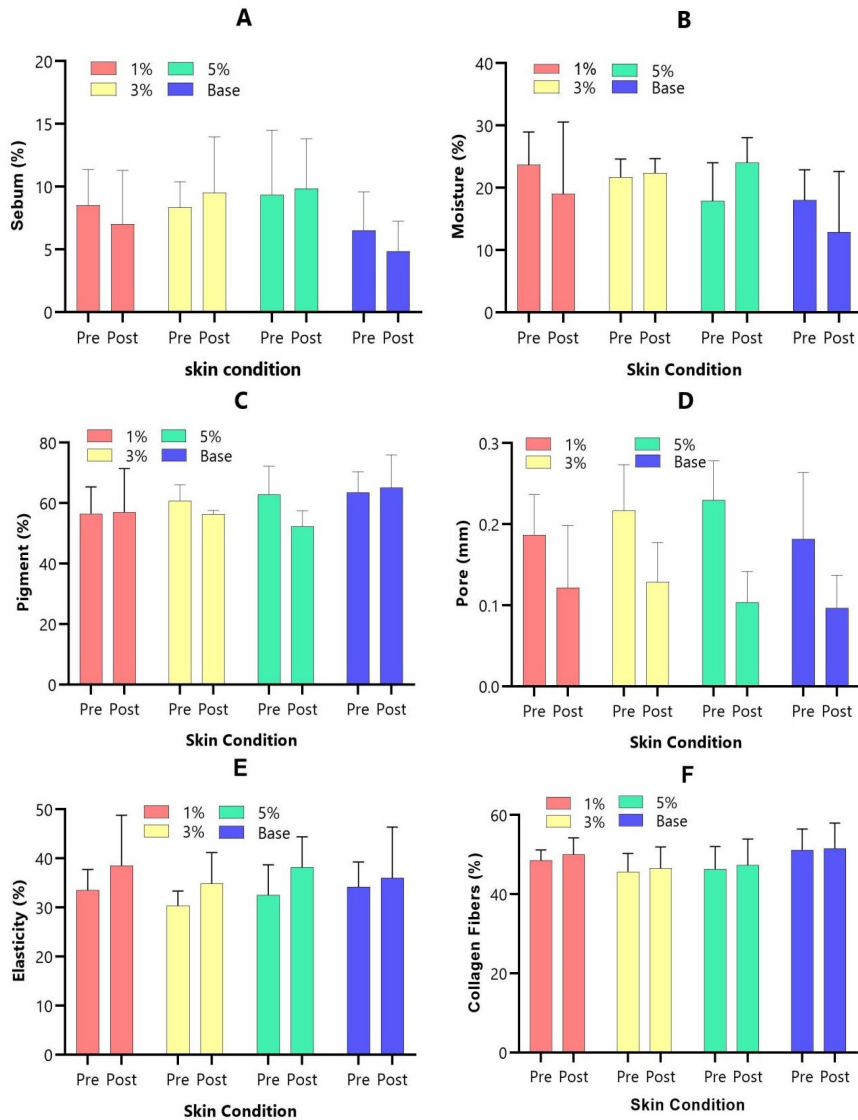


Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use (A)

Sebum, (B) Moisture, (C) Pigment, (D) Pore, (E) Elasticity, (F) Collagen Fibers

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

5 **Ririn Suharsanti<sup>1\*</sup>, Muhammad Ryan Radix Rahadhian<sup>1</sup>, Nining Sugihartini<sup>2</sup>, Endang Lukitaningsih<sup>3</sup>**

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10                                   **Abstract**

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

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

28                                   **Abstrak**

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

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

46 **Introduction**

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

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

Comment[L2]: The same as above

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

67 The total phenolic content of *Averrhoa bilimbi* fruits extract is  $19.8022 \pm 1.6696$  mg  
68 GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g and total flavonoids  
69  $24.7458 \pm 0.3291$  mg RE/g more than leaves ethanolic extract  $13.3126 \pm 0.5695$  mg RE/g. The  
70 SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can  
71 be described in the category of sunblock assessment with maximum protection. *Averrhoa*  
72 *bilimbi* fruit extract has an  $IC_{50}$  value of antioxidant activity of  $2.33 \pm 0.33$  mg/ml and  
73 tyrosinase inhibitor activity with an  $IC_{50}$  value of  $186.85 \pm 9.37$  mg/mL (Suharsanti et al.,  
74 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf  
75 samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa*  
76 *bilimbi* fruits contains flavonoids, alkaloids, saponins, and terpenoids and quantitatively has

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

### Methods

#### a. Material

93 *Averrhoa bilimbi* dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin,  
94 stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor,  
95 Indonesia), New Zealand Rabbits aged 12-16 weeks, Volunteers include subjects aged 19–  
96 25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State),  
97 skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

#### b. Extraction of *Averrhoa bilimbi*

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

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

#### c. Formula Preparation

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

Comment[L4]: citation

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

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

111 **d. Physical Characteristics of Anti-aging Cream**

112 ***Organoleptic Test***

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

115 ***Homogeneity Test***

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

120 ***pH Test***

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

124 ***Viscosity Test***

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

128 **e. Ethical Clearance**

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

135 **f. Irritation Test on test animals**

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

141 tests were also conducted on humans with the same treatment. Observe the presence of  
142 erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring  
143 System) according to table 2. Calculate the average score for each parameter at 24, 48, and  
144 72 hours (OECD, 2021). Irritation conditions were also observed during application of the  
145 cream to humans during the initial 3 days of use.

146 **g. Informed Consent**

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

157 **h. Human Trial of Anti-aging Cream**

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

168 **i. Analysis Data**

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

172 **Results and Discussion**

173 **a. Characteristics of Anti-aging Cream**

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

178 The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure  
179 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range.  
180 The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin  
181 and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing  
182 extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality  
183 requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus  
184 et al., 2024). So all cream preparation formulas have met the pH and viscosity quality  
185 requirements. The viscosity results for F1, F2, and F3 can be seen in Figure 1B.

186 **b. Irritation Test of Anti-aging Cream**

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

192 **c. Human Trial of Anti-aging Cream**

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

199 ***Skin Sebum***

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

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

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

### 217 **Skin Moisture**

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

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

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

### 245 **Skin Pigment**

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

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

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

Comment[L6]: The reason for what?

The sentence is repaired

*Averrhoa bilimbi* has a skin depigmentation effect because it contains tyrosinase inhibitors

### 264 **Skin Pores**

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

Comment[L7]: How long does it take?

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

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

### 279 **Skin Elasticity**

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

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

### 294 **Skin Collagen Fibers**

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

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

### Conclusion

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

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410 **Table 1.** *Averrhoa bilimbi* 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

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

Formula	Organoleptic			Homogeneity
	Smell	Form	Colour	
<b>Base</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	White	Homogeneous
<b>F1</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
<b>F2</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
<b>F3</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

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**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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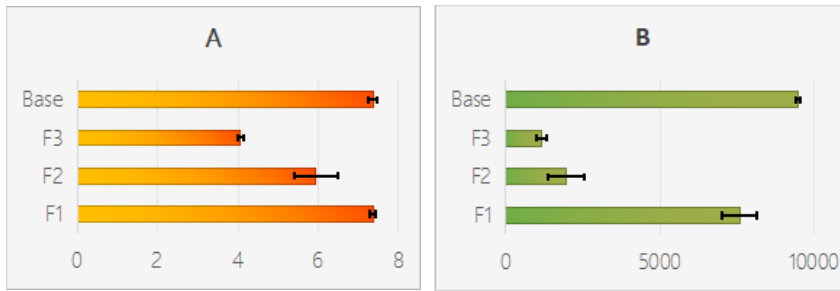


Figure 1. Results of pH (A) and Viscosity (B) tests on anti-aging cream

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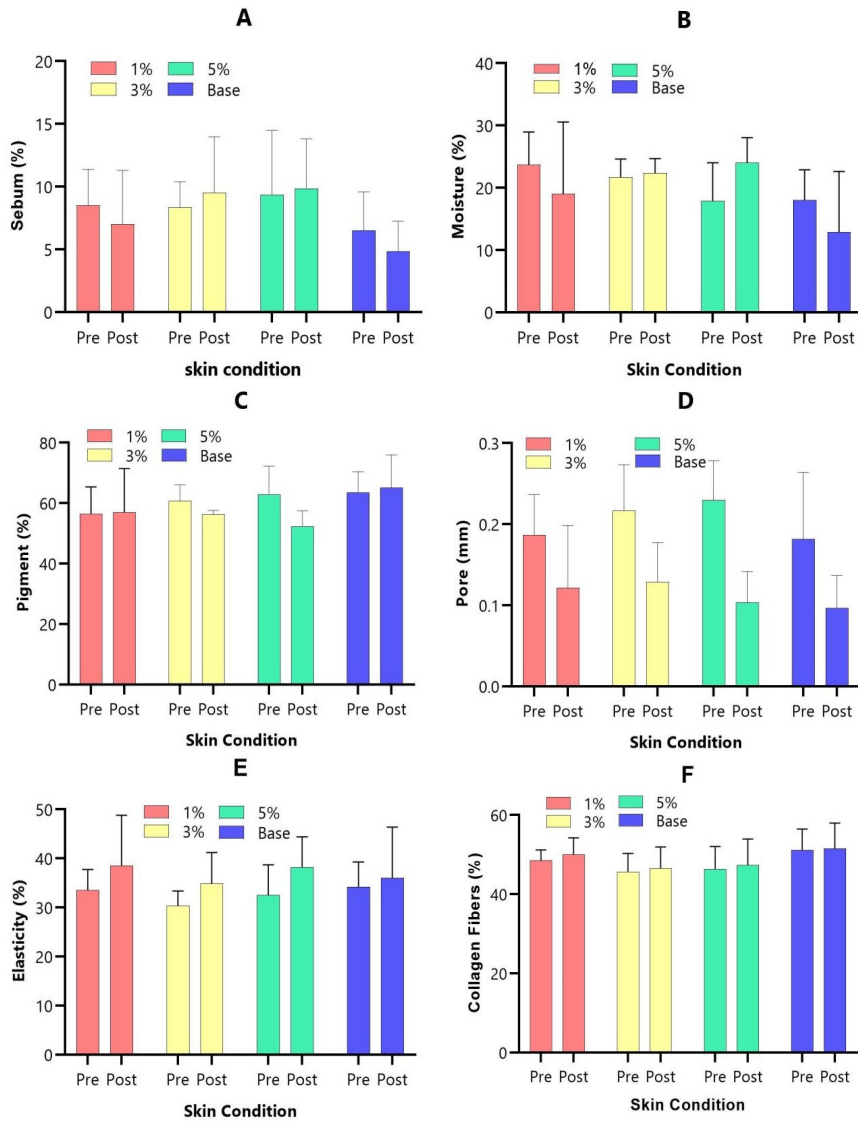


Figure 2. Skin Condition Against Anti-aging Parameters after 30 days of cream use (A)

Sebum, (B) Moisture, (C) Pigment, (D) Pore, (E) Elasticity, (F) Collagen Fibers

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

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11   **Abstract**

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

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

29   **Abstrak**

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

44 pengujian statistik t test pada masing-masing parameter anti-aging, krim F3 telah terbukti  
45 efektif memiliki potensi untuk memperkecil pori-pori kulit dan meningkatkan elastisitas kulit.

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

47

### Introduction

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

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

68 The total phenolic content of *Averrhoa bilimbi* fruits extract is  $19.8022 \pm 1.6696$  mg  
69 GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g and total flavonoids  
70  $24.7458 \pm 0.3291$  mg RE/g more than leaves ethanolic extract  $13.3126 \pm 0.5695$  mg RE/g. The  
71 SPF (Sun Protecting Factor) value of the ethanol extract concentration of 300 ppm fruit can  
72 be described in the category of sunblock assessment with maximum protection. *Averrhoa*  
73 *bilimbi* fruit extract has an  $IC_{50}$  value of antioxidant activity of  $2.33 \pm 0.33$  mg/ml and  
74 tyrosinase inhibitor activity with an  $IC_{50}$  value of  $186.85 \pm 9.37$  mg/mL (Suharsanti et al.,  
75 2019). Research on *Averrhoa bilimbi* has been conducted for wound healing but on leaf  
76 samples. Based on the results of the study qualitatively ethanol extract 70% *Averrhoa*

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

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

## 95 **Methods**

### 96 **a. Material**

97 *Averrhoa bilimbi* dried powder fruits, ethanol (Bratachem, Indonesia), TEA, glycerin,  
98 stearic acid, cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor,  
99 Indonesia), New Zealand Rabbits aged 12-16 weeks, Volunteers include subjects aged 19–  
100 25 years, pH meter (Hanna instrument, Indonesia), viscometer (Brookfield, United State),  
101 skin analyzer (Rista Clinic Beauty Care (Demak Regency, Central Java, Indonesia).

### 102 **b. Extraction of *Averrhoa bilimbi***

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

### 106 **c. Formula Preparation**

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

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

#### 116 **d. Physical Characteristics of Anti-aging Cream**

##### 117 ***Organoleptic Test***

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

##### 120 ***Homogeneity Test***

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

##### 125 ***pH Test***

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

##### 129 ***Viscosity Test***

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

#### 133 **e. Ethical Clearance**

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

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

141 **f. Irritation Test on test animals**

142 Irritation Test was conducted with male new zealand rabbits aged 12-16 weeks with the  
143 OECD method. The fur of the back and sides was shaved carefully  $\pm$  24 hours before  
144 application. The area is about 6 cm<sup>2</sup>. Apply 0.5 g of cream to each formula. Wrap the area  
145 with a semi-occlusive bandage (half-closed, but still allows air exchange). Observe the  
146 animal after 1 hour, 24 hours, 48 hours, and 72 hours directly to the open skin. Irritation  
147 tests were also conducted on humans with the same treatment. Observe the presence of  
148 erythema (redness) and edema (swelling) and write down the irritation score (Draize Scoring  
149 System) according to table 2. Calculate the average score for each parameter at 24, 48, and  
150 72 hours (OECD, 2021). Irritation conditions were also observed during application of the  
151 cream to humans during the initial 3 days of use.

152 **g. Informed Consent**

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

163 **h. Human Trial of Anti-aging Cream**

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

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

#### 174 **i. Analysis Data**

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

### 178 **Results and Discussion**

#### 179 **a. Characteristics of Anti-aging Cream**

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

184 The results of the pH test on anti-aging creams F1, F2, and F3 can be seen in Figure  
185 1A. The increase in pH value occurs as the extract increases but is still within a safe pH range.  
186 The requirements for a good pH preparation are 4.5 - 8.0 to match the natural pH of the skin  
187 and prevent irritation (Ariem et al., 2020; SNI, 1996). Viscosity also increases with increasing  
188 extract but remains within a safe pH range. Based on SNI 16-4399-1996, the viscosity quality  
189 requirements for sunscreen preparations (creams) are 2000 – 50.000 cPs (SNI, 1996; Firdaus  
190 et al., 2024). The appropriate viscosity will help the cream to be easily applied, spread  
191 evenly on the skin, and increase the absorption of active substances. So all cream  
192 preparation formulas have met the pH and viscosity quality requirements. The viscosity  
193 results for F1, F2, and F3 can be seen in Figure 1B.

#### 194 **b. Irritation Test of Anti-aging Cream**

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

#### 200 **c. Human Trial of Anti-aging Cream**

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

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

### 207 ***Skin Sebum***

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

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

### 228 ***Skin Moisture***

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

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

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

### 259 **Skin Pigment**

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

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

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

### 281 **Skin Pores**

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

290 F3 anti-aging cream is able to reduce the diameter of skin pores better than F1 and F2.  
291 Based on figure 2, F3 has the ability to shrink pores by up to 54.18%. The results of statistical  
292 tests with paired t-tests on each concentration with baseline (skin condition before being  
293 given anti-aging cream) show that anti-aging F2 and F3 cream has an effect on the condition  
294 of skin pores because sig <0.05 (different). This means that the concentration of the extract  
295 has an effect on skin pores. It can be concluded that *Averrhoa bilimbi* F2 and F3 creams have  
296 anti-aging activity in shrinking pores. Tannin compounds which are included in phenolics can  
297 be used as astringents. Astringents in plants are useful for shrinking pores (Puspitasari,  
298 2011).

**299 Skin Elasticity**

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

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

**318 Skin Collagen Fibers**

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

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

330

**Conclusion**

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

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**Table 1.** *Averrhoa bilimbi* 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

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

Formula	Organoleptic			Homogeneity
	Smell	Form	Colour	
<b>Base</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	White	Homogeneous
<b>F1</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
<b>F2</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
<b>F3</b>	<i>Averrhoa bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

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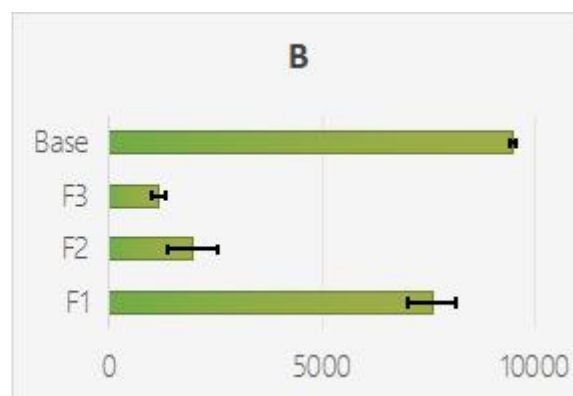
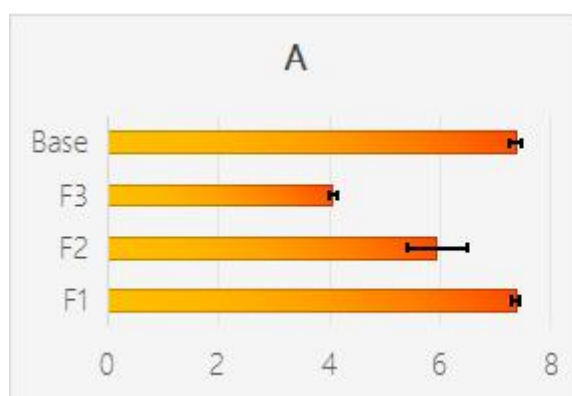
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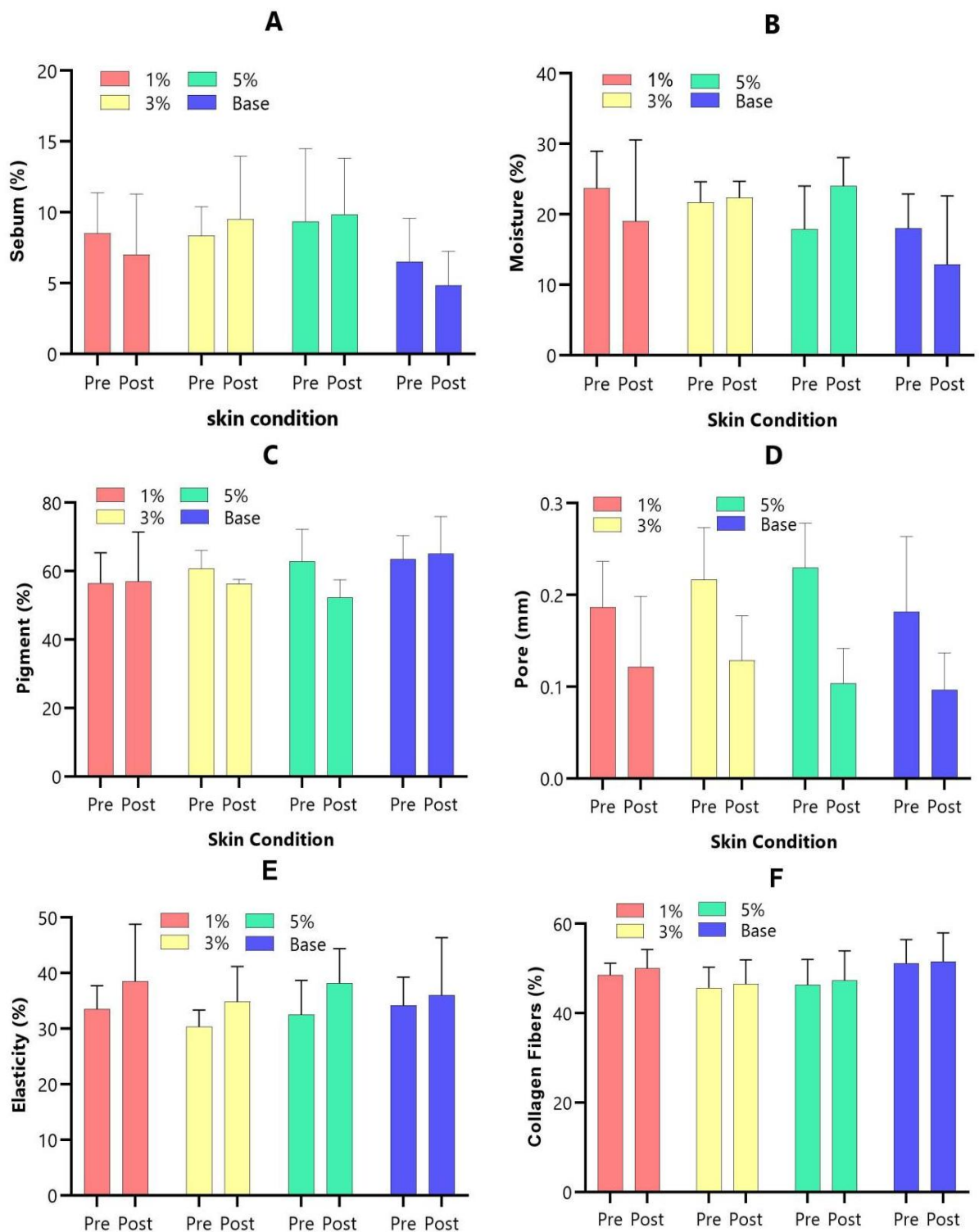
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**Figure 1.** Results of pH (A) and Viscosity (B) tests on anti-aging cream



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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

## **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**

<b>Editor or Reviewer Number</b>	<b>Original comments of the editor/reviewer</b>	<b>Reply by the author(s)</b>	<b>Changes done on page number and line number</b>
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 : <b>Photoaging</b>	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 <b>dried fruit powdered</b>	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: <b>Iskandar et al., 2016</b>	Page 3, line 107
3411	Should be eliminated because of double word	we rewrite as suggested  we have replaced with : <b>hormonal or other health problems</b>	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 : <b>Averrhoa bilimbi has a skin depigmentation effect because it contains thyronase inhibitors</b>	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  <b>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.</b>	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  <b><u>In animals: Irritation Test was conducted with male new zealand rabbits aged 12-16 weeks with the OECD method</u></b>  <u>In Human:</u> 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
		<p><b>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. .</b></p>	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	<p>We have written as suggested</p> <p>we have written the relationship of pH and viscosity characteristic tests to skin irritation and anti-aging effect</p> <p><b>pH :</b> 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).</p> <p><b>Viscosity :</b> 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.</p>	<p>Page 6, line184-186</p> <p>Page 6, line186-190</p>

**Note : Highlighted yellow for changes to response reviewer.**

1 **Anti-Aging Efficacy of *Averrhoa bilimbi* Fruit Extract Cream: A Human Clinical**  
2 **Trial**  
3 **Efektivitas Krim Ekstrak Buah *Averrhoa bilimbi* terhadap Parameter Penuaan**  
4 **Kulit: Studi Uji Klinis pada Manusia**

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11 **Abstract**

12 *Averrhoa bilimbi* is an Indonesian native plant known for its antioxidant, sunscreen, and  
13 tyrosinase inhibitory activities due to its polyphenol and flavonoid content. This study aimed  
14 to evaluate the anti-aging efficacy of a topical cream containing *A. bilimbi* fruit extract  
15 through a human clinical trial. The extract was obtained by maceration using 70% ethanol  
16 and formulated into a water-in-oil (W/O) cream at concentrations of 1% (F1), 3% (F2), and  
17 5% (F3). Each formulation was evaluated for its physical characteristics, including  
18 organoleptic properties, homogeneity, pH, and viscosity. Irritation tests were conducted on  
19 animals and human volunteers. A total of 24 human subjects applied the cream for 30 days.  
20 Skin condition parameters—sebum, moisture, pigmentation, pore size, elasticity, and  
21 collagen fibers—were assessed before and after treatment using a skin analyzer. The results  
22 showed that the F3 formulation produced the most notable improvements, including  
23 increased skin moisture and elasticity, and decreased pigmentation and pore size. Statistical  
24 analysis revealed significant improvements ( $p < 0.05$ ) in pore size and elasticity for the F3  
25 group. These findings indicate that the 5% *A. bilimbi* extract cream is a safe and effective  
26 anti-aging topical formulation.

27 **Keywords:** *Averrhoa bilimbi*, anti-aging cream, herbal cosmetics, clinical trial, skin elasticity,  
28 pore size

29 **Abstrak**

30 Belimbing wuluh (*Averrhoa bilimbi*) merupakan tanaman asli Indonesia yang mengandung  
31 senyawa aktif seperti polifenol dan flavonoid, serta memiliki aktivitas antioksidan, tabir  
32 surya, dan penghambat enzim tirosinase. Penelitian ini bertujuan mengevaluasi efektivitas  
33 krim yang mengandung ekstrak buah *A. bilimbi* dalam mengatasi tanda-tanda penuaan kulit  
34 melalui uji klinis pada manusia. Ekstrak buah diperoleh melalui maserasi dengan etanol 70%  
35 dan diformulasikan menjadi krim tipe air dalam minyak (A/M) dengan konsentrasi 1% (F1),  
36 3% (F2), dan 5% (F3). Evaluasi karakteristik sediaan meliputi uji organoleptik, homogenitas,  
37 pH, dan viskositas. Uji iritasi dilakukan pada hewan dan manusia. Sebanyak 24 subjek  
38 relawan menggunakan krim selama 30 hari. Evaluasi parameter kulit dilakukan sebelum dan  
39 sesudah penggunaan dengan bantuan *skin analyzer*, yang mencakup kadar sebum,  
40 kelembaban, pigmentasi, diameter pori, elastisitas, dan serat kolagen. Hasil menunjukkan  
41 bahwa formula F3 memberikan hasil paling optimal, dengan peningkatan kelembaban dan  
42 elastisitas kulit, serta penurunan pigmentasi dan diameter pori secara bermakna ( $p < 0,05$ ).  
43 Oleh karena itu, krim ekstrak buah *A. bilimbi* konsentrasi 5% berpotensi sebagai sediaan  
44 antipenuaan yang aman dan efektif.

## Editor's comment

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

### Introduction

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

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

61 *Averrhoa bilimbi*, a plant native to Indonesia, has shown potential in this regard. The  
62 fruit of *A. bilimbi* contains higher levels of phenolics and flavonoids compared to its leaves,  
63 with total phenolic content reaching  $19.80 \pm 1.67$  mg GAE/g and total flavonoid content of  
64  $24.75 \pm 0.33$  mg RE/g (Suharsanti dkk., 2019) . In addition, the fruit extract demonstrates sun  
65 protection factor (SPF) values indicative of maximum protection at 300 ppm concentration,  
66 antioxidant activity with an  $IC_{50}$  of  $2.33 \pm 0.33$  mg/mL, and tyrosinase inhibitory activity with  
67 an  $IC_{50}$  of  $186.85 \pm 9.37$  mg/mL.

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

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

76

## Editor's comment

77

### Materials and Methods

#### 78 **Materials**

79 Dried *Averrhoa bilimbi* fruits were used as the plant material. Other ingredients  
80 included ethanol 70% (Bratachem, Indonesia), triethanolamine (TEA), glycerin, stearic acid,  
81 cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor, Indonesia).  
82 Experimental animals were male New Zealand rabbits aged 12–16 weeks. Human subjects  
83 were healthy male and female volunteers aged 19–25 years. Equipment included a pH  
84 meter (Hanna Instruments, Indonesia), a viscometer (Brookfield, USA), and a skin analyzer  
85 (Rista Clinic Beauty Care, Demak Regency, Central Java, Indonesia).

#### 86 **Extraction of *A. bilimbi***

87 A total of 200 g of dried powdered *A. bilimbi* fruit was extracted by maceration with  
88 70% ethanol for 3 days at room temperature. The extract was then concentrated using a  
89 rotary evaporator at 60°C and 100 rpm to obtain a viscous crude extract (Rahardian dkk.,  
90 2019).

#### 91 **Cream Formulation**

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

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

Ingredient	F1 (%)	F2 (%)	F3 (%)
<i>A. bilimbi</i> 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

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

103

#### 104 Evaluation of Cream Characteristics

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

111

#### 112 Ethical Clearance

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

#### 117 Irritation Test

118 The irritation test was conducted in accordance with OECD guidelines (OECD, 2021). On  
119 each rabbit, a 6 cm<sup>2</sup> area of shaved dorsal skin was treated with 0.5 g of cream and covered  
120 using a semi-occlusive bandage. Observations for erythema and edema were made at 1, 24,  
121 48, and 72 hours, and results were scored using the Draize method (**Table 2**). The same  
122 procedure was applied to human subjects, with additional daily observation during the first  
123 three days of product use.

124 **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

125

#### 126 **Informed Consent**

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

132

#### 133 **Human Trial**

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

#### 140 **Analysis Data**

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

145

### 146 **Results and Discussion**

#### 147 **Characteristics of the Anti-Aging Cream**

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

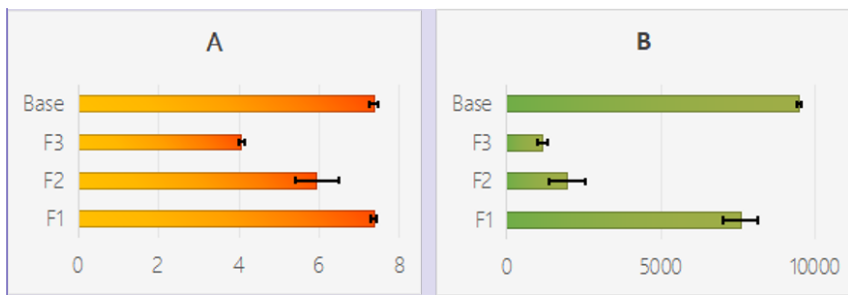
152 **Table 3.** Organoleptic and Homogeneity Characteristics of Anti-aging Cream

Formula	Smell	Form	Color	Homogeneity
Base	<i>A. bilimbi</i> fruit	Semi solid	White	Homogeneous

**Editor's comment**

F1	<i>A. bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
F2	<i>A. bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
F3	<i>A. bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

153  
 154 The pH values of all formulations increased slightly with increasing extract  
 155 concentration but remained within the acceptable topical range of 4.5–6.5, indicating  
 156 compatibility with skin physiology (Figure 1A). Viscosity was also observed to increase with  
 157 extract concentration but remained within the quality standard of 2,000–50,000 cPs for  
 158 cream preparations (Figure 1B), complying with SNI 16-4399-1996. Optimal viscosity  
 159 enhances cream spreadability and skin absorption.



Comment[WN2]: Satuan viskositas harus ada, bisa dikirimkan untuk data excelnya? Supaya kami bisa perbaiki dengan lebih baik

160  
 161 **Figure 1.** pH (A) and Viscosity (B) of Anti-aging Cream Formulations

162  
 163 **Irritation Test Results**

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

167 **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

168  
 169 **Human Trial: Anti-Aging Parameters**

#### Editor's comment

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

172 Sebum levels slightly increased in F2 and F3 groups, likely due to the higher oil content  
173 in W/O formulations. However, these changes were statistically non-significant ( $p > 0.05$ ).

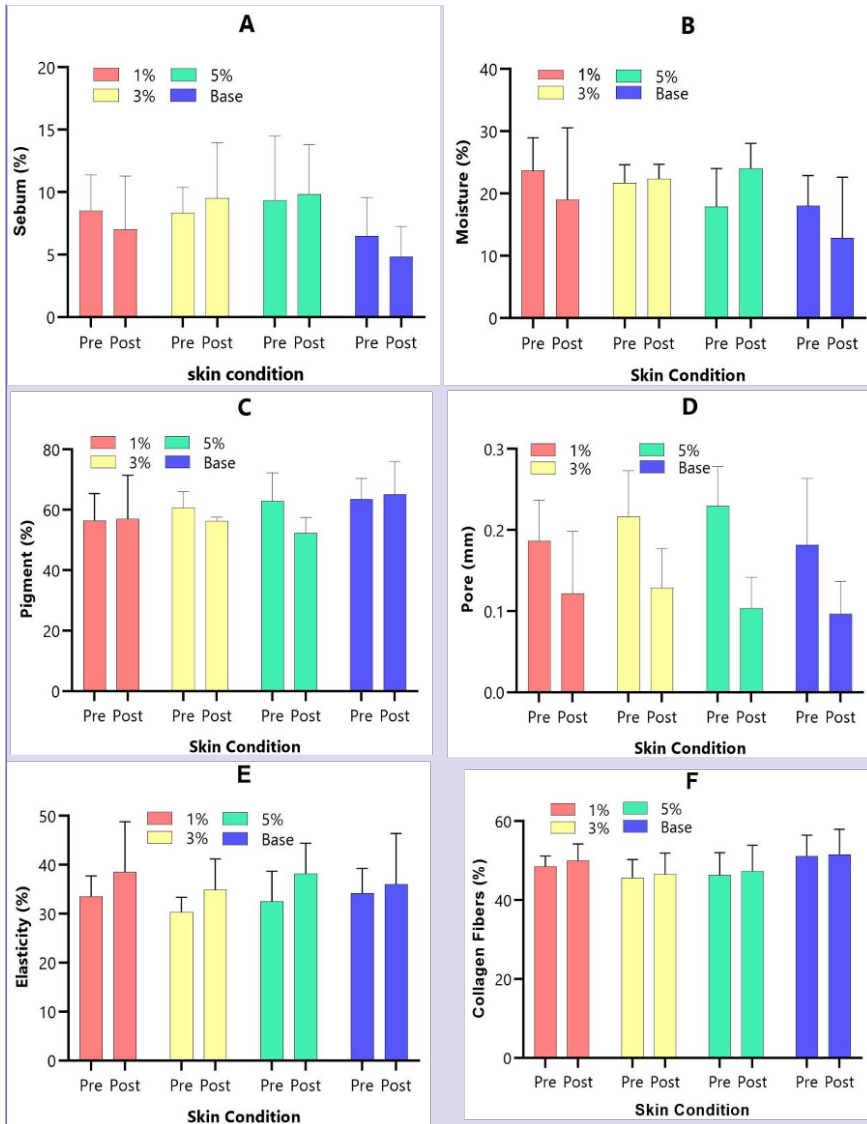
174 Excessive sebum can obstruct pores, but the increase here did not exceed normal levels and  
175 may be counteracted by the formula's other active properties.

176 F3 showed the highest increase in skin hydration (22.34%) after 30 days of  
177 application. Nonetheless, this improvement was not statistically significant ( $p > 0.05$ ).

178 Moisturizing effects of flavonoid-rich extracts have been documented, but a longer duration  
179 may be needed to observe significant change (Rasul & Akhtar, 2012; Sugihartini, 2017).

180 F3 also exhibited the most effective pigmentation reduction (15.08%). However,  
181 statistical analysis did not support significance ( $p > 0.05$ ). This result aligns with previous  
182 studies that reported visible depigmentation only after 8 weeks of application (Khan dkk.,  
183 2014).

Editor's comment



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!

184  
 185 **Figure 2.** Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture,  
 186 (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers  
 187 Significant reduction in pore diameter was observed in both F2 and F3 groups ( $p <$   
 188 0.05), with F3 achieving the greatest reduction (54.18%). This effect may be attributed to

### Editor's comment

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

191 Skin elasticity improved significantly in the F3 group ( $p < 0.05$ ), with an increase of  
192 16.85%. This result suggests that *A. bilimbi* extract may contribute to collagen preservation  
193 or synthesis. Improved elasticity correlates well with the observed pore shrinkage, indicating  
194 restored dermal integrity (Jadoon dkk., 2015).

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

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

205

206

### Conclusion

207 Based on the findings from this study, the cream formulation containing 5% *Averrhoa*  
208 *bilimbi* extract (F3) demonstrated the most significant anti-aging effects, particularly in  
209 reducing skin pore diameter and improving skin elasticity, both of which were statistically  
210 significant ( $p < 0.05$ ). Although other parameters such as moisture content, pigmentation,  
211 and collagen fiber density showed positive trends, they did not reach statistical significance  
212 within the 30-day application period. The results support the use of *A. bilimbi* fruit extract in  
213 topical formulations for anti-aging purposes. Further studies with longer treatment  
214 durations and higher extract concentrations are recommended to strengthen and expand  
215 these findings.

216

217

### Acknowledgment

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### Editor's comment

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222 Tinggi/PKPT). We also thank the volunteers and medical personnel at Rista Clinic Beauty  
223 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.

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

### 47 Introduction

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

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

61 *Averrhoa bilimbi*, a plant native to Indonesia, has shown potential in this regard. The  
62 fruit of *A. bilimbi* contains higher levels of phenolics and flavonoids compared to its leaves,  
63 with total phenolic content reaching  $19.80 \pm 1.67$  mg GAE/g and total flavonoid content of  
64  $24.75 \pm 0.33$  mg RE/g (Suharsanti dkk., 2019) . In addition, the fruit extract demonstrates sun  
65 protection factor (SPF) values indicative of maximum protection at 300 ppm concentration,  
66 antioxidant activity with an  $IC_{50}$  of  $2.33 \pm 0.33$  mg/mL, and tyrosinase inhibitory activity with  
67 an  $IC_{50}$  of  $186.85 \pm 9.37$  mg/mL.

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

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

76

77

**Materials and Methods****Materials**

79 Dried *Averrhoa bilimbi* fruits were used as the plant material. Other ingredients  
 80 included ethanol 70% (Bratachem, Indonesia), triethanolamine (TEA), glycerin, stearic acid,  
 81 cetyl alcohol, stearyl alcohol, nipagin, and nipasol (CV. Setia Jaya Distributor, Indonesia).  
 82 Experimental animals were male New Zealand rabbits aged 12–16 weeks. Human subjects  
 83 were healthy male and female volunteers aged 19–25 years. Equipment included a pH  
 84 meter (Hanna Instruments, Indonesia), a viscometer (Brookfield, USA), and a skin analyzer  
 85 (Rista Clinic Beauty Care, Demak Regency, Central Java, Indonesia).

**Extraction of *A. bilimbi***

87 A total of 200 g of dried powdered *A. bilimbi* fruit was extracted by maceration with  
 88 70% ethanol for 3 days at room temperature. The extract was then concentrated using a  
 89 rotary evaporator at 60°C and 100 rpm to obtain a viscous crude extract (Rahardhian dkk.,  
 90 2019).

**Cream Formulation**

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

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

Ingredient	F1 (%)	F2 (%)	F3 (%)
<i>A. bilimbi</i> extract	1	3	5
Stearic Acid	24	24	24
Cetyl Alcohol	4	4	4
Stearyl alcohol	2	2	2
Nipasol	0,5	0,5	0,5

Nipagin	0,5	0,5	0,5
Glycerin	28	28	28
TEA	2	2	2
Distilled water	ad 100	ad 100	ad 100

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

### 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<sup>2</sup> 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

125

126 **Informed Consent**

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

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 138 elasticity, and collagen fiber density. Daily self-assessments were also recorded using a  
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146 **Results and Discussion**

147 **Characteristics of the Anti-Aging Cream**

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 151 organoleptic and homogeneity observations are summarized in **Table 3**.

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

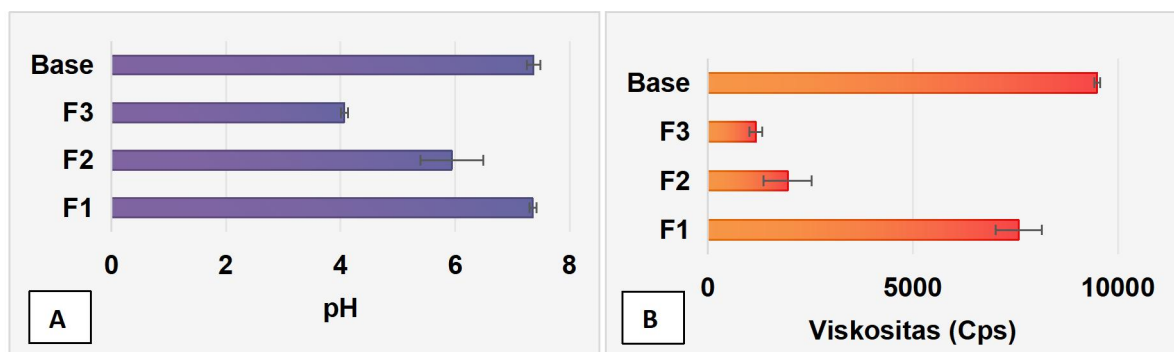
Formula	Smell	Form	Color	Homogeneity
Base	<i>A. bilimbi</i> fruit	Semi solid	White	Homogeneous

F1	<i>A. bilimbi</i> fruit	Semi solid	Light yellow	Homogeneous
F2	<i>A. bilimbi</i> fruit	Semi solid	Deep Yellow	Homogeneous
F3	<i>A. bilimbi</i> fruit	Semi solid	Deep yellow	Homogeneous

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

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

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164 **Irritation Test Results**

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

168 **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

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170 **Human Trial: Anti-Aging Parameters**

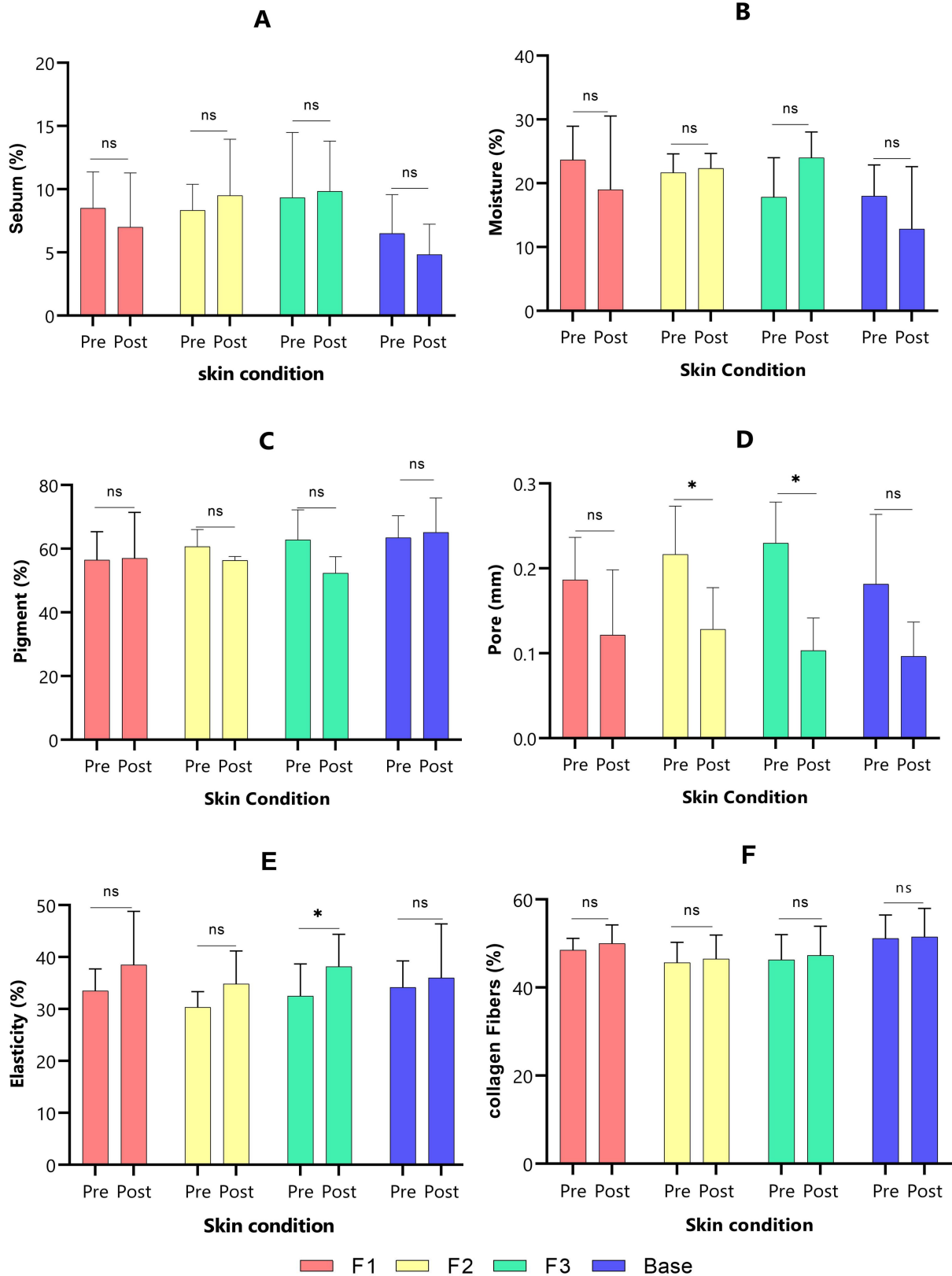
176 Anti-aging efficacy was assessed by comparing skin parameters before and after 30  
177 days of cream application. The skin condition improvements are visualized in Figure 2.

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

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

186 F3 also exhibited the most effective pigmentation reduction (15.08%). However,  
187 statistical analysis did not support significance ( $p > 0.05$ ). This result aligns with previous  
188 studies that reported visible depigmentation only after 8 weeks of application (Khan dkk.,  
189 2014).

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**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  $\pm$  SD (n=6), ns = no significant, \* = significant (p<0,05) with t test

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263 Significant reduction in pore diameter was observed in both F2 and F3 groups ( $p <$   
264 0.05), with F3 achieving the greatest reduction (54.18%). This effect may be attributed to  
265 phenolic compounds, including tannins, which act as astringents and help tighten skin pores  
266 (Puspitasari, 2011).

267 Skin elasticity improved significantly in the F3 group ( $p < 0.05$ ), with an increase of  
268 16.85%. This result suggests that *A. bilimbi* extract may contribute to collagen preservation  
269 or synthesis. Improved elasticity correlates well with the observed pore shrinkage, indicating  
270 restored dermal integrity (Jadoon dkk., 2015).

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

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

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### Conclusion

283 Based on the findings from this study, the cream formulation containing 5% *Averrhoa*  
284 *bilimbi* extract (F3) demonstrated the most significant anti-aging effects, particularly in  
285 reducing skin pore diameter and improving skin elasticity, both of which were statistically  
286 significant ( $p < 0.05$ ). Although other parameters such as moisture content, pigmentation,  
287 and collagen fiber density showed positive trends, they did not reach statistical significance  
288 within the 30-day application period. The results support the use of *A. bilimbi* fruit extract in  
289 topical formulations for anti-aging purposes. Further studies with longer treatment  
290 durations and higher extract concentrations are recommended to strengthen and expand  
291 these findings.

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## Research Article

Doi: <https://doi.org/10.29244/jji.v10i1.YYY>

# 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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skin elasticity  
pore size

#### Kata kunci:

Belimbing wuluh  
*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  $19.80 \pm 1.67$  mg GAE/g and total flavonoid content at  $24.75 \pm 0.33$  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  $2.33 \pm 0.33$  mg/mL, and tyrosinase inhibition with an  $IC_{50}$  of  $186.85 \pm 9.37$  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 (%)
<i>A. bilimbi</i> extract	1	3	5
Stearic Acid	24	24	24
Cetyl Alcohol	4	4	4
Stearyl alcohol	2	2	2
Nipasol	0.5	0.5	0.5
Nipagin	0.5	0.5	0.5
Glycerin	28	28	28
TEA	2	2	2
Distilled water	ad 100	ad 100	ad 100

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

### 2.4. 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

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

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

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<sup>2</sup> shaved dorsal skin area of each rabbit was treated with 0.5 g of cream under a semi-occlusive bandage. Observations for erythema and edema were recorded at 1, 24, 48, and 72 hours, using the Draize scoring method (Table 2). The same protocol was applied to human volunteers, with additional daily monitoring during the first three days of use.

**Table 2.** Irritation Score (Draize Scoring System)

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

### 2.7. 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.

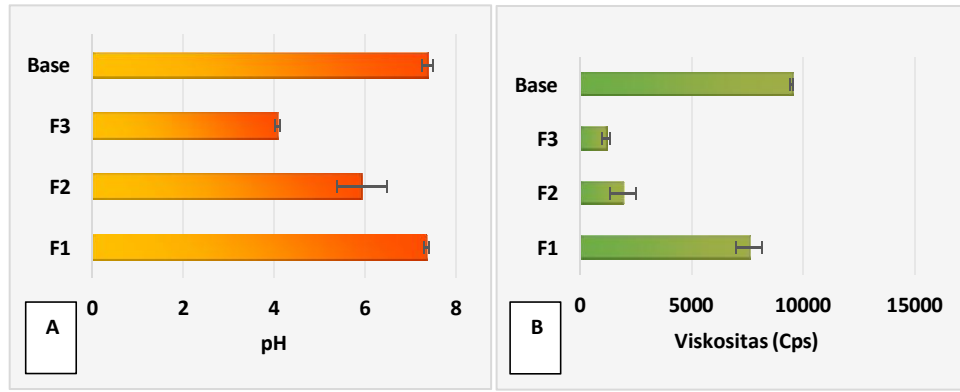


Figure 1. pH (A) and Viscosity (B) of Anti-aging Cream Formulations

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).

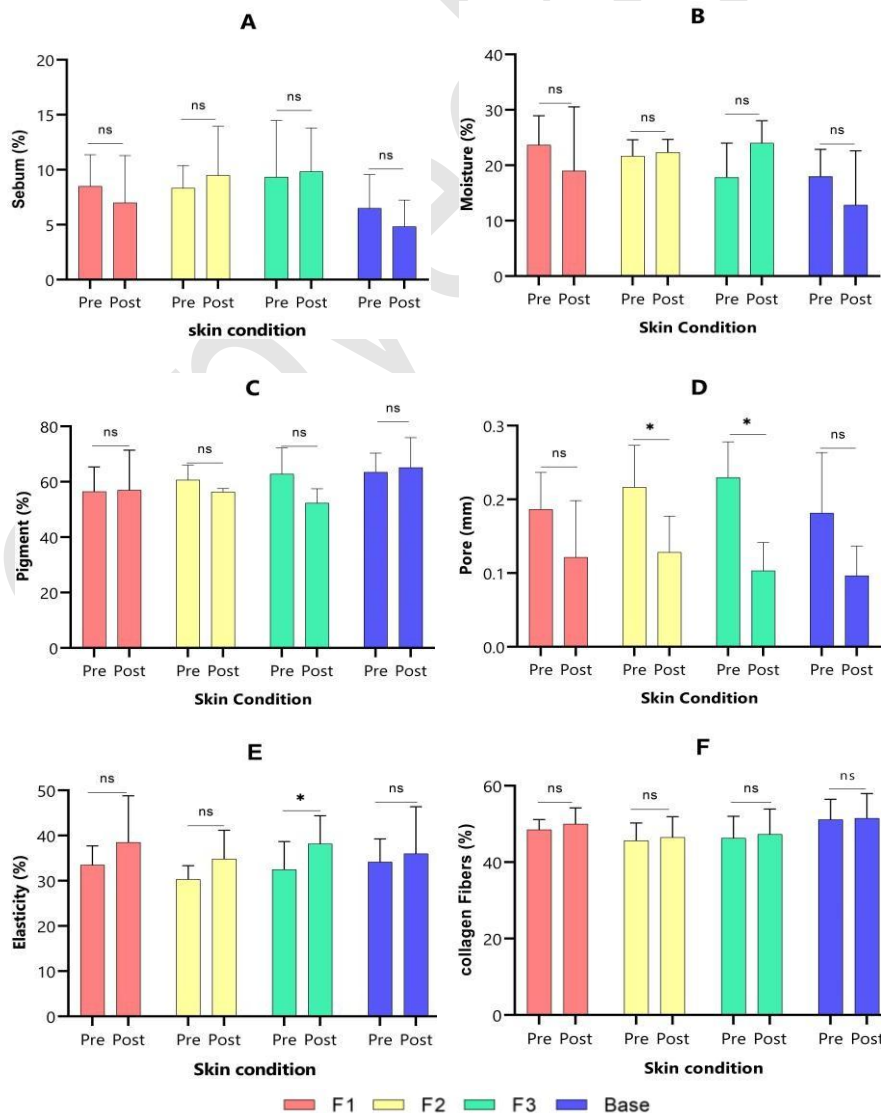


Figure 2. Changes in Skin Parameters After 30 Days of Application: (A) Sebum, (B) Moisture, (C) Pigment, (D) Pore Size, (E) Elasticity, and (F) Collagen Fibers, Mean ± SD (n=6), ns = no significant, \* = significant ( $p < 0,05$ ) with t test.

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.

#### 5. 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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## Research Article

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

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

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### ABSTRACT

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

### ABSTRAK

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



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## 1. INTRODUCTION

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

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

*Averrhoa bilimbi*, a tropical plant native to Indonesia, has demonstrated significant potential in skin care applications. Its fruit contains notably higher levels of phenolic and flavonoid compounds compared to its leaves, with total phenolic content reported at  $19.80 \pm 1.67$  mg GAE/g and total flavonoid content at  $24.75 \pm 0.33$  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  $2.33 \pm 0.33$  mg/mL, and tyrosinase inhibition with an  $IC_{50}$  of  $186.85 \pm 9.37$  mg/mL.

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

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

## 2. METHOD

### 2.1. Materials

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

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

### 2.2. Extraction of *A. bilimbi*

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

### 2.3. Cream Formulation

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

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

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

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

### 2.4. Ethical Clearance

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

### 2.5. Irritation Test

Irritation tests were conducted in compliance with OECD guidelines (OECD, 2021). A 6 cm<sup>2</sup> shaved dorsal skin area of each rabbit was treated with 0.5 g of cream under a semi-occlusive bandage. Observations for erythema and edema were recorded at

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

**Table 2.** Irritation Score (Draize Scoring System)

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

**2.6. Informed Consent**

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

**2.7. Human Trial**

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

**2.8. Analysis Data**

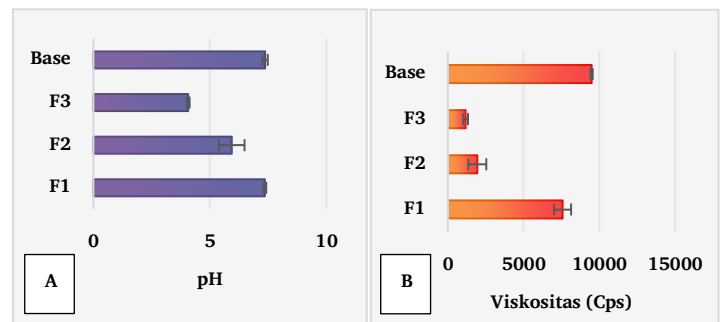
The data obtained from skin analysis before and after the 30-day treatment were analyzed using paired t-tests for each group. A p-value of < 0.05 was considered statistically significant. The formulation yielding the most statistically significant improvement was considered the most effective.

**3. RESULTS AND DISCUSSION**

**3.1. Characteristics of The Anti-Aging Cream**

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

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



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

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

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

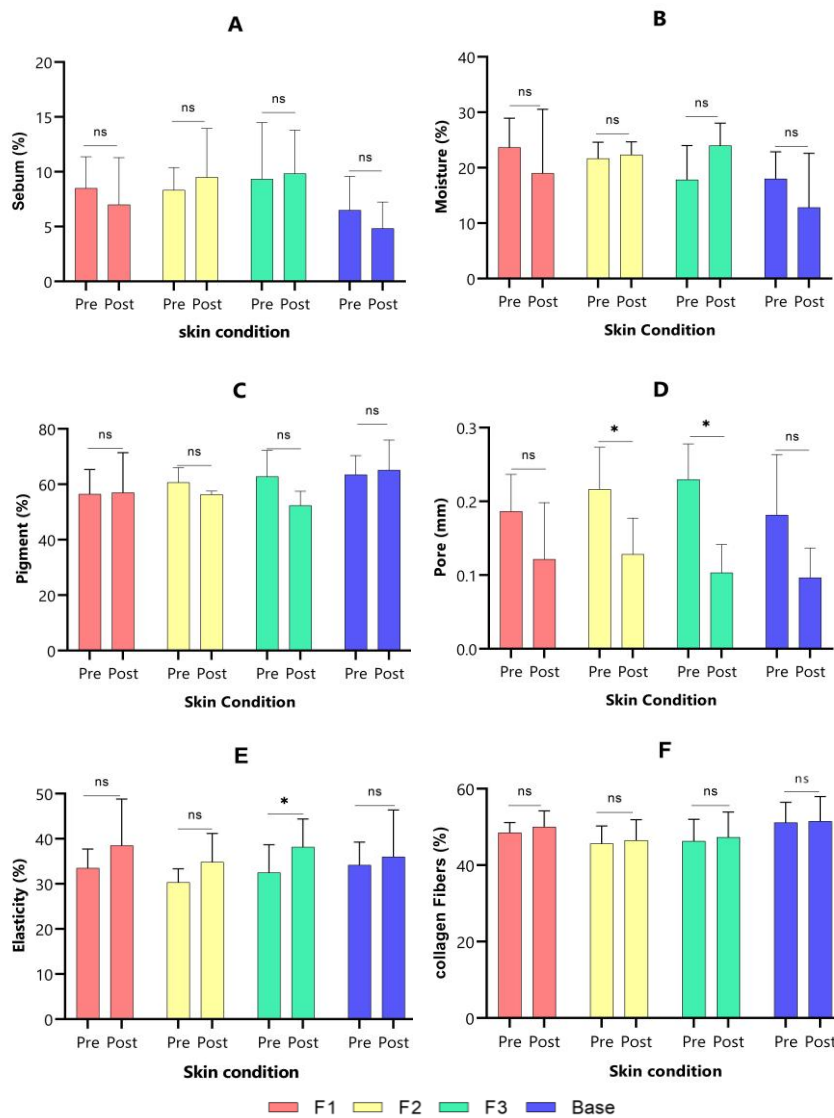
Formula	Animal Irritation Index	Human Irritation Index
Base	0	0
F1	0	0

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

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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

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