International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 5, Number 3, September 2026, *Page. 3186 - 3199*
Email : editorijhess@gmail.com

Optimization And Stability Testing Of Avocado Leaf (*Persea americana*) Extract Soap Formulated With A Combination Of VCO, Palm Oil, And Olive Oil Using The Simplex Lattice Design Method

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Abstract

Continuous exposure to free radicals such as sunlight, air pollution, and cigarette smoke can cause dry, dull skin, skin lesions, and premature aging. Therefore, it is necessary to utilize natural ingredients with antioxidant properties to address these issues. Avocado (Persea americana) leaves are known to contain various secondary metabolites such as flavonoids, saponins, alkaloids, and tannins, which have high antioxidant activity. The use of these natural ingredients in solid soap offers a safe and effective solution to protect the skin from the negative effects of free radicals. This study was conducted to determine the optimum formula and combination of VCO, palm oil, and olive oil in the manufacture of avocado leaf extract soap. Stability and antioxidant tests were conducted. Optimization using the Simplex Lattice Design (SLD) method aims to determine the precise concentrations of ingredients to obtain a formula with optimal physical properties and a consumer-acceptable response. This study was optimized using the Simplex lattice design method, antioxidant testing using the DPPH method, and stability testing using a cycling test. The results showed that avocado leaf extract has very strong antioxidant activity, with an IC_{50} value of 16.127 µg/mL. Formula optimization using Simplex lattice design resulted in an optimal formula with 10% VCO, 30% palm oil, and 10% olive oil. Solid soap was formulated with avocado leaf extract into three formulas. Formula 3, with a 10% extract concentration, achieved the highest IC_{50} value of 17.878 µg/mL. Thus, avocado leaf extract has very strong antioxidant content.

Keywords: Antioxidant, Solid Soap,SLD, Avocado

INTRODUCTION

Indonesia has a tropical climate that causes most people to sweat easily, especially during activities involving direct exposure to sunlight. This environment fosters the rapid growth and proliferation of bacteria on the human body. Bar soap is a common daily necessity for cleansing the body and maintaining skin health; it comes into direct contact with the skin to remove dirt and disease-causing germs while restoring skin freshness (Dwiputri et al., 2022).

Soap functions as a cleansing agent composed of a surfactant or a mixture of surfactants. It can take the form of a solid bar, a soft paste, or a liquid, and it produces lather during use. Its primary function is to help remove dirt and microorganisms adhering to the skin. Soap is produced through saponification—a reaction between glyceryl tripalmitate and a base—yielding sodium salts of long-chain fatty acids (the soap itself) and glycerol as a byproduct. Fatty acid sources typically include vegetable oils or animal fats. Sodium hydroxide (NaOH) is used to produce solid soap, whereas potassium hydroxide (KOH) is used for liquid soap (Wuryandari & Pamella, 2025).

Organic soap is a popular choice among consumers because it is made from organic ingredients and is free from synthetic chemical additives; it can also serve as an exfoliating body soap. These natural ingredients enable organic soap to absorb and retain moisture, keeping the skin hydrated and soft. Additionally, it is eco-friendly, safe for all skin types, helps treat back acne, is suitable for dry and sensitive skin, and helps maintain a youthful, healthy complexion by reducing signs of aging (Yudanti et al., 2026).

Natural ingredients suitable for producing organic solid soap include Virgin Coconut Oil, palm oil, and olive oil. Virgin Coconut Oil contains 46% lauric acid, which moisturizes the skin; it is highly valued in soap making for its excellent cleansing and lathering properties. Furthermore, the resulting solid soap is resistant to rancidity; the oil retains its beneficial properties and remains stable for up to

two years without going rancid. Palm oil contains a significant amount of palmitic acid—approximately 44.3%. In soap formulations, palmitic acid increases the soap's hardness, provides a smooth texture, and ensures a stable lather. Its high palmitic acid content makes palm oil more resistant to oxidation and rancidity compared to other oils. Olive oil consists of 70–80% fatty acids, which enhance the absorption of other active ingredients. Its high oleic acid content is particularly beneficial for the skin, as it helps remove dead skin cells and moisturizes flaky skin. Additionally, olive oil can help reduce the appearance of scars and firm up wrinkled skin (Dwiputri et al., 2022).

Antioxidants are molecules that slow down or prevent oxidation, a chemical reaction capable of generating free radicals. Continuous exposure to free radical sources—such as sunlight, air pollution, certain medications, and cigarette smoke—can lead to dry, dull skin, skin lesions, and premature aging (Hanifah et al., 2021). Consequently, utilizing natural ingredients rich in antioxidants is essential to address these issues. Avocado leaves are known to contain various secondary metabolites—such as flavonoids, saponins, alkaloids, and tannins—that exhibit high antioxidant activity. Flavonoids act as free radical scavengers and protect skin cells from oxidative damage, thereby helping to maintain the integrity of the skin's protective barrier. Protecting this skin layer plays a crucial role in maintaining the skin's natural moisture and preventing excessive water loss from the skin's surface (Novasari et al., 2021). Yadi and Pertiwi (2024) reported that avocado leaves possess significant antioxidant activity; antioxidant testing of the avocado leaf extract yielded an IC_{50} value of 49.659 ppm, a result classified as strong antioxidant activity.

Previous studies have demonstrated the potential of avocado leaves as a skin-moisturizing agent. Research by Novasari et al. (2021) showed that lotion formulations containing 3% and 5% avocado leaf extract significantly increased skin moisture, as measured by a skin moisture analyzer. This increase in moisture is attributed to the presence of flavonoids and polyphenols, which enhance water retention in the stratum corneum.

Optimization using the Simplex Lattice Design (SLD) method aims to determine the precise concentrations of ingredients required to achieve a formula with optimal physical properties and consumer-acceptable characteristics. The SLD method allows for the optimization of formulas involving varying ingredient compositions, thereby yielding an optimal formula that meets the desired physical specifications (Purwanto et al., 2024).

Based on this background, the researchers aim to investigate the effectiveness of a soap formulation derived from avocado leaf extract. The optimal formula in this study will be determined using the Simplex Lattice Design (SLD) method, involving the optimization of three ingredient components.

RESEARCH METHODS

The research employed an experimental method involving the production of solid soap formulations. The study aimed to determine the optimal formula by varying combinations of Virgin Coconut Oil (VCO), palm oil, and olive oil in solid soap containing avocado leaf (*Persea americana*) extract. A Simplex Lattice Design (SLD) with three mixture components was used for optimization. Each formulation combination derived from this design was processed into soap, and the physical properties—specifically pH, foam height, moisture content, and foam stability—were evaluated. The test data were analyzed to identify the oil composition that yielded the best soap characteristics.

RESULTS AND DISCUSSION

Research Results

Determination

The results of the plant identification indicate that the plants used in this study are indeed avocados (Persea americana) from the Lauraceae family. The identification results can be seen in Appendix 3.

Drying and Powder Making

Avocado leaves (*Persea americana*): Four kilograms of avocado leaves underwent wet sorting, washing, and drying (sun-drying while covered with a black cloth). The dried crude drug material was then blended into a fine powder and sieved using a 40-mesh screen, yielding 1,700 grams of powder. The yield of the dried crude drug material was 42.5%, while the yield of the powder was 70.58%. Calculations regarding these yields can be found in Appendices 8 and 9.

Standardization of Crude Drugs

Standardization of the *simplisia* (crude drug material) is conducted to ensure its quality, safety, and compliance with applicable requirements before it is used as a raw material in the extraction process. The objective of standardization is to guarantee that the *simplisia* is of high quality, thereby enabling the production of a high-quality extract. The parameters tested in this study included loss on drying, moisture content determination, and ash content determination of avocado leaf (*Persea americana*) *simplisia*. The results of the *simplisia* standardization are presented in the table.

Determination of Loss on Drying

Based on Table 4.1 above, the loss on drying test for avocado leaf (*Persea americana*) powder—conducted by placing the sample in an oven, performing three replicates, and calculating the average—yielded a loss on drying value of 3.92%. The calculation for loss on drying can be found in Appendix 13.

Table 1 Loss on Drying of Powder

Replicate	Powder loss on drying (%)	Mean ± SD
Replicate 1	3,96	
Replicate 2	4,2	3,92 ± 0,30
Replicate 3	3,6	

Determination of Moisture Content

The determination of moisture content was performed on avocado (*Persea americana*) leaf powder using a moisture balance, with three replicates. The moisture content of the avocado (*Persea americana*) leaf powder was found to be 5.59%.

Table 2 Powder Moisture Content

Replicate	Powder moisture content (%)	Mean ± SD
Replicate 1	7,29	
Replicate 2	4,86	5,59 ± 1,49
Replicate 3	4,63	

Determination of Ash Content

Based on Table 4.3, the ash content of the powder was determined to be 4.7%. The determination was performed by placing a weighed amount of the powder into a porcelain crucible and subjecting it to ignition in a muffle furnace for 2 hours.

Table 3 Ash content of the powder

Replicate	Ash Content (%)	Mean ± SD
Replicate 1	6,15	
Replicate 2	7,9	6,70 ± 1,04
Replicate 3	6,05	

Extraction

Extraction was performed using the maceration method with 96% ethanol at a 1:10 ratio; 147 grams of thick extract were obtained from 500 grams of avocado leaf powder. The extraction process yielded a recovery rate of 5.8%.

Extract Standardization

Determination of water content

The moisture content of the avocado leaf (*Persea americana*) extract was determined using a moisture balance, with three replicates performed. The determined moisture content was 5.8%. Calculations regarding the extract's moisture content can be found in Appendix 16.

Table 4 Extract moisture content

Replicate	Moisture Content (%)	Mean ± SD
Replicate 1	5,1	
Replicate 2	6,3	6,11 ± 0,93
Replicate 3	6,95	

Determination of ash content

The determination of ash content was performed on avocado leaf (*Persea americana*) extract via a combustion process using a muffle furnace at a temperature of 600°C. The result of the ash content determination, following three replicates, was 7.96%.

Table 5 Ash content of the extract

Replicate	Ash Content (%)	Mean ± SD
Replicate 1	7,7	
Replicate 2	7,85	7,967 ± 0,341
Replicate 3	8,35	

Ethanol-Free Test for the Extract

Test results indicate that the avocado leaf extract does not exhibit the characteristic ester odor associated with the ethanol solvent.

Phytochemical Screening

Test-tube analyses were conducted to detect alkaloids, saponins, tannins, and flavonoids. Phytochemical screening was performed to identify the chemical compounds present in the avocado leaf extract. The results of the phytochemical screening for the avocado leaf extract are presented in Table 6:

Table 6 Phytochemical Screening Test

Secondary metabolite		Reagent	Positive result (+)	Notes
Flavonoid		HCL+ Mg	Positive	A yellowish-orange color forms
Saponin		Air panas+HCl	Positive	A stable foam forms
Alkaloid		Dragendroff	Positive	An orange or light brown precipitate forms
Tanin		$FeCl_3$ 5%	Positive	A greenish-black color forms

Formula Optimization of Avocado Leaf Extract Solid Soap

Optimization of the solid soap formula containing avocado leaf (*Persea americana*) extract was conducted using the Simplex Lattice Design (SLD) method with Design-Expert-13 software. The optimization aimed to determine the optimal concentration combination of olive oil, VCO, and palm oil. The proportions of olive oil, VCO, and palm oil were entered into the Design-Expert-13 software using the Simplex Lattice Design, resulting in the following 11 formulas:

Table 7 Comparative composition of olive oil, VCO, and palm oil in the simplex lattice design.

Nama bahan	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Olive oil	10	20	16,66	13,33	30	10	23,33	10	10	13,33	20
VCO	20	10	16,66	23,33	10	30	13,33	30	10	13,33	20
Palm oil	20	20	16,66	13,33	10	10	13,33	10	30	23,33	10

Formula optimization was based on parameters including pH, foam height, and moisture content. The physical quality results were then entered into Design-Expert 13 software.

pH test

The pH test is a chemical parameter used to determine whether the resulting soap is acidic or alkaline. An excessively alkaline pH can cause skin irritation, such as itching, lesions, and dryness (Fitri et al., 2023). The pH test results for the avocado leaf (*Persea americana*) extract soap are presented in Table 8.

Table 8 Results of pH Test Evaluation

Formulas	pH
F1	9,76
F2	9,94
F3	10,53
F4	10,51
F5	10,64
F6	10,79
F7	10,75
F8	10,52
F9	10,65
F10	10,4
F11	10,35

Foam Height Test

The measurement of foam height aims to assess the foaming capacity of the organic solid soap. The ideal foam height range for solid soap is 13–220 mm (Sulistiana et al., 2023). The results of the foam height test are presented in Table 9.

Table 9 Foam Height Test Evaluation Results

Formulas	Tinggi Busa
F1	6.6
F2	5.4
F3	6
F4	6.5
F5	5.2
F6	6.4
F7	4.06
F8	5.8
F9	6.8
F10	6.2
F11	4.6

Moisture Content Test

Moisture content was measured using a moisture balance. The maximum allowable moisture content for solid bath soap products is 15% (SNI 3532:2016).

Table 10 Moisture Content Test Evaluation Results

Formulas	Kadar Air
F1	8.27
F2	10.66
F3	9.79
F4	10.27
F5	10.54
F6	8.63

F7	10.53
F8	10.4
F9	7.56
F10	7.79
F11	9.11

Determination of the Optimum Formula

The optimum formula for avocado leaf (*Persea americana*) extract soap was determined using physical test parameters, including pH, foam height, and moisture content. The results for the optimum formula can be viewed in the solution table within the Simplex lattice design application by establishing criteria for each response. The criteria for formula optimization are presented in Table 11.

Table 11 Criteria for the Optimum Formula

Name	Goal	Lower limit	Uper limit
Olive oil	In range	10%	50%
VCO	In range	10%	50%
Palm oil	In range	10%	50%
pH	In range	9	11
Moisture content	In range	1%	15%
Foam height	Maximize	4,06 cm	6,8 cm

The optimization of avocado leaf extract soap formulations using Simplex Lattice Design (SLD) aims to determine the optimal composition of ingredients, thereby producing a solid soap with physical properties that meet quality standards.

Table 12 Soap Quality Based on SNI 3532:2016 Standards

No	Paraneter	Standar	Formulas 1	Formula s 2	Formulas 3
1.	am lemak bebas % (b/b)	Maks. 2,5	0,42	0,38	0,32
2.	ahan tak larut dalam etanol %(b/b)	Maks. 10,0	3,41	6,02	6,54
3.	Lemak total, % (b/b)	Min. 60,0	8,37	11,00	13,00

Stability Test

Based on the research conducted, the results regarding the physical organoleptic quality stability of the preparation are presented in Table 13.

Table 13 Organoleptic Test Results

Cycle	Formulas	Color	Odor	Form
0	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
1	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
Cycle	**Formulas**	Color	Odor	Shape
2	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested

	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
3	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
4	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
5	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested
6	F0	White	characteristic soap scent	Congested
	F1	Brown	characteristic extract scent	Congested
	F2	Dark brown	characteristic extract scent	Congested
	F3	Blackish-brown	characteristic extract scent	Congested

The results regarding the physical stability of the formulation's pH are presented in Table 14.

Table 14 pH Test Results

Cycle	Formulas	R1	R2	R3	Mean ± SD
0	F0	10,20	10,22	10,23	10,22 ± 0,02
	F1	10,17	10,40	10,24	10,24 ± 0,14
	F2	10,35	10,38	10,30	10,30 ± 0,12
	F3	10,25	10,32	10,31	10,31 ± 0,06
1	F0	9,92	9,85	9,81	9,86 ± 0,06
	F1	9,82	9,94	9,89	9,88 ± 0,06
	F2	9,77	9,71	9,77	9,75 ± 0,03
	F3	9,83	9,89	9,90	9,87 ± 0,04
2	F0	9,37	9,32	9,40	9,36 ± 0,04
	F1	9,28	9,55	9,60	9,48 ± 0,17
	F2	9,29	9,54	9,48	9,44 ± 0,13
	F3	9,51	9,49	9,45	9,48 ± 0,03
3	F0	9,40	9,41	9,55	9,45 ± 0,09
	F1	9,44	9,47	9,45	9,45 ± 0,02
	F2	9,43	9,44	9,58	9,48 ± 0,08
	F3	9,41	9,45	9,48	9,45 ± 0,04
4	F0	9,30	9,28	9,35	9,31 ± 0,04
	F1	9,22	9,27	9,24	9,24 ± 0,03
	F2	9,23	9,25	9,22	9,23 ± 0,02

	F3	9,24	9,23	9,28	9,25 ± 0,003
5	F0	9,25	9,27	9,23	9,25 ± 0,02
	F1	9,40	9,29	9,26	9,32 ± 0,07
	F2	9,31	9,30	9,31	9,31 ± 0,01
	F3	9,28	9,37	9,29	10,31 ± 0,08
6	F0	9,16	9,20	9,20	9,19 ± 0,02
	F1	9,16	9,16	9,18	9,17 ± 0,01
Cycle	**Formulas**	**R1**	**R2**	**R3**	**Mean ± SD**
	F2	9,12	9,11	9,15	9,13± 0,02
	F3	9,16	9,10	9,24	9,17 ± 0,07

Table 15 Foam Height Stability Test

Cycle	Formulas	R1	R2	R3	Mean ± SD
0	F0	6	6,4	6	6,13 ± 0,23
	F1	6	6	5,5	5,83 ± 0,29
	F2	6	5,5	6,5	6,00 ± 0,50
	F3	5	6,5	6,5	6,00 ± 0,87
1	F0	6,5	6	6,5	6,33 ± 0,29
	F1	6	6,4	6	6,13 ± 0,23
	F2	6	6,5	5,9	6,13 ± 0,32
	F3	5,5	6	6	5,83 ± 0,29
2	F0	6	6	5,8	5,93 ± 0,12
	F1	5,5	6	6	5,83 ± 0,29
	F2	6	6,5	6,5	6,33 ± 0,29
	F3	5	5,5	5	5,17 ± 0,29
3	F0	6	6	6	6,00 ± 0,00
	F1	6	6	5,9	5,97 ± 0,06
	F2	6	6,2	6	6,07 ± 0,12
	F3	6,5	5,5	6	6,00 ± 0,50
4	F0	6	6	5,6	5,87 ± 0,23
	F1	6	5	5	5,33 ± 0,58
	F2	5,5	5	5	5,17 ± 0,29
	F3	5,5	5	5	5,17± 0,29
5	F0	6	5,8	6	5,93 ± 0,12
	F1	6	6,5	5,5	6,00 ± 0,50
	F2	5,4	5,5	6	5,63 ± 0,32
	F3	6,5	6	5,5	6,00 ± 0,50
6	F0	6	6	6,4	6,13 ± 0,23
	F1	6,2	6,5	6	6,23 ± 0,25
	F2	5,8	5,5	5	5,43 ± 0,40
	F3	6	5,5	5,5	5,67 ± 0,29

Based on the research conducted, the results regarding the physical quality stability (moisture content) of the preparation are presented in Table 17.

Table 16 Moisture Content Stability Test

Cycle	Formulas	R1	R2	R3	Mean ± SD
0	F0	9,55	9,54	9,14	9,41 ± 0,23
	F1	8,67	9,30	9,23	9,07 ± 0,35
	F2	9,48	9,32	9,71	9,50 ± 0,20
	F3	9,73	9,89	9,58	9,73 ± 0,16
1	F0	9,29	9,02	9,45	9,25 ± 0,22
	F1	8,59	8,79	9,50	8,96 ± 0,48
	F2	9,96	8,09	8,25	8,77 ± 1,04
	F3	9,12	9,20	9,49	9,27 ± 0,19
2	F0	9,56	9,20	9,12	9,29 ± 0,23

	F1	8,70	8,56	8,30	8,52 ± 0,20
	F2	9,10	8,30	8,41	8,60 ± 0,43
	F3	8,52	8,56	9,12	8,37 ± 0,30
3	F0	8,73	8,56	8,60	8,63 ± 0,09
	F1	7,79	7,70	6,77	7,42 ± 0,56
	F2	6,74	6,15	6,03	6,31 ± 0,38
	F3	7,42	6,46	6,50	6,79 ± 0,54
4	F0	7,60	7,83	7,92	7,78 ± 0,17
	F1	6,13	6,06	6,49	6,23 ± 0,23
	F2	6,07	7,62	7,79	7,16 ± 0,95
	F3	6,01	7,04	6,70	6,58 ± 0,52
5	F0	7,40	7,555	7,37	7,44 ± 0,10
	F1	6,32	6,46	7,40	6,73 ± 0,59
	F2	6,15	6,74	6,77	6,55 ± 0,35
	F3	6,70	7,34	6,03	6,69 ± 0,66
6	F0	7,03	7,20	6,94	7,06 ± 0,13
	F1	5,15	6,07	6,13	5,78 ± 0,55
	F2	6,06	6,49	5,90	6,15 ± 0,31
	F3	5,94	7,02	5,50	6,153 ± 0,782

Antioxidant Activity Assay

The antioxidant activity assay in this study was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Prior to the assay, the maximum wavelength and operating time were determined to establish optimal measurement conditions, thereby ensuring accurate and precise results.

Determination of Maximum Wavelength

The determination of the maximum wavelength aimed to identify the wavelength yielding the highest absorbance value for the DPPH solution. This maximum wavelength served as the basis for absorbance measurements during the antioxidant activity assay to ensure result accuracy.

Determination of Operating Time

The operating time was determined to establish the duration required for the reaction between the DPPH solution and the reference standard to reach a stable state.

Determination of Vitamin C Content

Vitamin C was used as a positive control in the DPPH antioxidant activity assay. This assay aimed to evaluate the capacity of Vitamin C to scavenge free radicals, expressed in terms of percentage inhibition and IC_{50} values.

Determination of Avocado Leaf Extract Content

The antioxidant activity of avocado leaf (*Persea americana*) extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to determine the extract's free radical scavenging capacity.

Table 17 Antioxidant Test Results for Avocado Extract

Wavelength	Absorbansi	ppm	R1	R2	R3	Average	% inhibisi	IC 50 ppm
		2	0,503	0,498	0,491	0,497	21,360	
		4	0,470	0,468	0,481	0,473	25,158	
516	0,632	6	0,453	0,452	0,453	0,434	31,329	16,127
		8	0,427	0,423	0,427	0,425	32,753	
		10	0,392	0,396	0,396	0,394	37,658	

Based on the antioxidant activity test results for the avocado leaf (*Persea americana*) extract, a linear regression analysis was performed between extract concentration and inhibition percentage to derive the regression equation and determine the IC_{50} value.

Assay of Formula F0

Antioxidant activity testing for Formula F0 was conducted using the DPPH method to assess the base formula's ability to scavenge free radicals.

Assay of Formula F1

Antioxidant activity testing for Formula F1 was conducted to assess the ability of the solid soap formulation containing 5% avocado leaf (*Persea americana*) extract to scavenge DPPH free radicals.

Assay of Formula F2

Antioxidant activity testing for Formula F2 was conducted to assess the ability of the solid soap formulation containing 7.5% avocado leaf (*Persea americana*) extract to scavenge DPPH free radicals.

Discussion

Plant Determination

Plant determination was conducted at the Traditional Health Service Unit (UPF) Laboratory of Dr. Sardjito General Hospital, located at the Tlogodringo Aromatic Garden, Tawangmangu, Karanganyar Regency, Central Java. The determination results indicated that the crude drug material (*simplisia*) used was avocado leaf (*Persea americana*) from the Lauraceae family. Determination aims to identify the plant—a crucial step in research to ascertain and confirm the family and species of a sample (Handayani et al., 2022). The determination results can be found in Appendix 2.

Drying and Preparation of Avocado Leaf (*Persea americana*) Powder

Drying is a method used to preserve plant materials by reducing their moisture content (Handayani et al., 2022). The purpose of the drying process is to enable long-term storage and halt enzymatic processes, thereby preventing quality degradation or spoilage of the crude drug material (Handoyo et al., 2020). The avocado leaves were dried using the sunlight method, with the material covered by a black cloth. Drying using direct sunlight is the most economical and easiest method, as it requires neither specialized techniques nor extensive equipment (Fahmi et al., 2019).

Standardization of Crude Drug Material (Simplicia)

Determination of Powder Drying Loss

The determination of drying loss for the powdered crude drug was conducted using an oven at 105°C for 30 minutes until a constant weight was achieved. This determination aims to establish the maximum limit for the amount of substances that evaporate or are lost during the drying process (Kusumawati & Kusumawati, 2023). The results of the powder drying loss determination are presented in Table 4.1.

The drying loss for the avocado leaf crude drug in this study was 3.92%, which meets the standard parameter requirement of less than 10% (Depkes RI, 2008).

Determination of Powder Moisture Content

The determination of the *simplisia* (crude drug) moisture content was performed using a moisture balance. Testing the moisture content aims to reduce the water level in the *simplisia* to prevent fungal growth. Establishing this moisture content value is crucial for setting a maximum limit on water content, as high water levels can serve as a medium for bacterial and fungal proliferation (Depkes RI, 2008).

Based on the table above, the moisture content of the *simplisia* was found to be 5.59%, indicating that it met the standard parameter requirements. According to the *Farmakope Herbal Indonesia* (2008), the moisture content must not exceed 10%.Determination of Powder Ash Content

The ash content test aims to provide an overview of the internal and external mineral content resulting from the entire process, from the initial stages through to the formation of the *simplisia*. Ash content determination was performed by weighing 2 g of bay leaf powder and placing it into a crucible that had been previously ignited and weighed. Subsequently, the powder was heated on a hot plate, followed by heating in a muffle furnace at 600°C for four hours until all carbonized material (charcoal) was consumed. After cooling, the average value from three replicates was calculated to be 4.7%, which meets the acceptable ash content standard of no more than 10% (Dayanti et al., 2023).

Extraction

The extraction process for avocado leaves was carried out using the maceration method. Maceration was selected due to several advantages: the equipment and operation are simple,

operational costs are relatively low, extraction occurs at room temperature without heating, and it effectively extracts chemical compounds from the *simplisia*. The solvent used for this maceration was 96% ethanol at a 1:10 ratio. The choice of 96% ethanol was based on its safety, ease of evaporation, and ability to dissolve a wide range of substances—whether polar, semi-polar, or non-polar—as well as its capacity to optimally extract flavonoid and phenolic compounds (Kusumawati & Kusumawati, 2023).

A 500 g sample of avocado leaf powder was extracted using 5 L of 96% ethanol over a period of 5 days. The resulting liquid extract was filtered using filter paper. The maceration product was then concentrated using a rotary evaporator at a temperature of 40–60°C and further thickened in a water bath until a viscous extract was obtained. The yield of the viscous extract was 147 g, representing a yield percentage of 29.4%. An extract yield is considered good if it exceeds 10% (Saerang et al., 2023).

Extract Standardization

Determination of Extract Moisture Content

The moisture content of the extract was determined using a moisture balance. Moisture content testing on the *simplicia* (dried plant material) was conducted to reduce moisture levels and prevent fungal growth. Establishing this moisture value is crucial for setting a maximum limit on water content, as high moisture levels can serve as a medium for bacterial and fungal proliferation (Ministry of Health RI, 2008). The moisture balance test was performed at 105°C for 15 minutes, yielding an average moisture content of 5.8% for the extract. This result indicates that the avocado leaf (*Persea americana*) extract complies with the standard parameter, which requires a moisture content of no more than 10% (Ministry of Health of the Republic of Indonesia, 2017). Determination of Extract Ash Content: The ash content test aims to provide an overview of the internal and external mineral content resulting from the entire process, from the initial stage through to the formation of the *simplicia*. Ash content was determined by weighing 2 g of bay leaf powder and placing it into a crucible that had been previously ignited and weighed. The powder was then heated on a hot plate, followed by treatment in a muffle furnace at 600°C for four hours until all carbonized material was consumed. After cooling, the average value from three replicates was calculated to be 7.967%, meeting the standard requirement for acceptable ash content—specifically, a maximum of 10% (Dayanti et al., 2023).

Ethanol-Free Test for Extract

The purpose of the ethanol-free test was to ensure that the concentrated extract was pure and free from ethanol. The test was conducted by placing the extract into a test tube, adding H2SO4 and CH3COOH, and then heating the mixture. The results showed that the avocado leaf extract produced no ester odor, indicating it was ethanol-free; the absence of ethanol is confirmed by the lack of an ester smell upon heating following the addition of acetic acid and sulfuric acid (Tivani et al., 2021).

Phytochemical Screening Test

Phytochemical screening was conducted to identify secondary metabolites present in avocado leaf extract (*Persea americana*). The secondary metabolites qualitatively tested were saponins, tannins, alkaloids, and flavonoids. The study revealed positive results for flavonoids, alkaloids, tannins, and saponins in the avocado leaf extract; these findings align with the research of Putri et al. (2023). Identification of the chemical constituents in avocado leaves yielded a positive (+) result for flavonoids, indicated by the formation of a yellowish-orange color following the addition of HCl and Mg reagents. The addition of HCl serves to hydrolyze flavonoids into their aglycone forms, specifically through the cleavage of O-glycosyl groups; the glycosyl group is subsequently replaced by an H+ ion from the acid, driven by the latter's electrophilic nature. A reduction process using Mg and concentrated HCl produces a complex compound characterized by a red or orange color. As phenolic compounds, flavonoids are characterized by the presence of multiple -OH groups; this polar nature facilitates their extraction using ethanol—a solvent that is also polar due to its hydroxyl group—thereby enabling the formation of hydrogen bonds. Flavonoids are commonly found in various fruits,

vegetables, and beverages, offering biochemical benefits and antioxidant activity (Rahmah et al., 2024).

Optimization of Solid Soap Formulations

Formula optimization was conducted using a simplex lattice design with Design-Expert software (version 13) to compare the concentrations of VCO, palm oil, and olive oil. The parameters evaluated during optimization were pH, foam height, and moisture content. The optimization aimed to determine the optimal solid soap formulation; the resulting data demonstrated the software's capability to meet requirements based on specific end-product criteria (Istiharoh et al., 2022). The physical test results were subsequently analyzed using Design-Expert version 13, with specific criteria—such as minimization or maximization—established by the researchers for each formulation response.

Antioxidant Activity Assay

Measurement of Maximum Wavelength

Prior to testing the antioxidant activity of avocado leaf extract using the DPPH method, the maximum wavelength was determined. This step aimed to identify the wavelength yielding maximum absorbance, thereby ensuring that subsequent absorbance measurements could be conducted with high sensitivity and precision. The maximum wavelength was determined by scanning the DPPH solution across the 400–800 nm range using a UV-Vis spectrophotometer.

The measurements yielded a maximum absorbance value of 0.635 at a wavelength of 516 nm. This result indicates that 516 nm is the maximum wavelength for the DPPH solution; consequently, this wavelength was used for all subsequent antioxidant activity assays.

The DPPH method was selected due to its ability to measure antioxidant activity quickly and simply. The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay evaluates antioxidant activity based on the capacity to scavenge free radicals. The DPPH solution was incubated for 30 minutes before the maximum wavelength was measured (Pharmauho, 2020).

Operating Time Determination Results

Prior to conducting the antioxidant activity assay using the DPPH method, the operating time (OT) was determined. This step aimed to establish the time required for the reaction between the DPPH solution and the sample to reach a stable state. Determining the operating time is crucial because absorbance readings taken before the reaction stabilizes can yield inaccurate antioxidant activity values. The operating time associated with a stable absorbance value—specifically 0.513—was found to be between the 2nd and 6th minutes.

Results of Vitamin C Content Determination

Vitamin C is used as a positive control in antioxidant activity assays due to its excellent natural free-radical scavenging ability, relative safety, and lack of toxicity. It functions as a secondary antioxidant by scavenging free radicals and inhibiting free-radical chain reactions; consequently, it is frequently used as a reference standard in DPPH-based antioxidant activity assays (Rohmah et al., 2022). In this study, the antioxidant activity of Vitamin C was assessed using the DPPH method across a concentration range of 2, 4, 6, 8, and 10 ppm. Each solution was reacted with a 100 ppm DPPH solution and incubated for a duration determined by a preliminary "operating time" test. Following incubation, absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm, with measurements performed in triplicate. The results indicated that increasing the Vitamin C concentration led to a decrease in absorbance values and a corresponding increase in the percentage of inhibition. This demonstrates that higher Vitamin C concentrations provide a greater number of antioxidant molecules capable of donating electrons or hydrogen atoms to DPPH radicals, thereby neutralizing a larger quantity of free radicals. The color change of the DPPH solution from purple to pale yellow serves as an indicator that the free-radical scavenging reaction has occurred (Rodiatullah et al., 2023). Subsequently, a linear regression curve plotting Vitamin C concentration against percentage inhibition was generated using Excel to determine the IC_{50} value. The IC_{50} value represents the concentration of a compound required to inhibit 50% of DPPH free radicals. Calculations yielded an IC_{50} value of 10.440 ppm for Vitamin C. This value falls into the "very strong" antioxidant category,

as it is below the 50 ppm threshold. A lower IC_{50} value indicates a higher capacity for a compound to scavenge free radicals.

CONCLUSION

Based on the research conducted on the optimization of a solid soap formulation, the antioxidant activity of avocado leaf extract, and stability testing, the following conclusions can be drawn:

1. The optimization results in this study identified a proportion of 10% VCO, 30% palm oil, and 10% olive oil as the optimal formula determined using Design-Expert software.
2. Avocado leaf (*Persea americana*) extract exhibits antioxidant activity with an IC_{50} value of 16.127 µg/mL, indicating very strong antioxidant activity.
3. Solid soap formulations containing avocado leaf (*Persea americana*) extract demonstrated antioxidant activity with IC_{50} values of 21.222 µg/mL for Formula 1 (5% concentration), 20.642 µg/mL for Formula 2 (7.5% concentration), and 17.878 µg/mL for Formula 3 (10% concentration); thus, it can be concluded that all three formulas possess very strong antioxidant activity.

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