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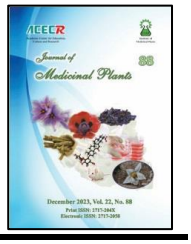

Research Article

Anti-inflammatory and anti-aging activity of hydrogel with active ingredient *Phyllantus emblica* L. fruit nanosimplicia

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ABSTRACT

Background: *Phyllantus emblica* contains quite high levels of vitamin C as an antioxidant, premature aging and anti-inflammatory. Simplicia is formulated into a hydrogel because it has good dispersion power on the skin. **Objective:** This research aims to develop a hydrogel formula with the active ingredient *Phyllantus emblica* L. Fruit nanosimplicia as an anti-inflammatory and anti-aging. **Methods:** Nano simplicia is made using a high-energy ball mill and characterized through phytochemical analysis, particle size analysis, Fourier transform infrared spectroscopy, and scanning electron microscopy. Hydrogels are formulated with simplicia concentrations (2, 4, 6 %). Assessment of hydrogels encompasses organoleptic evaluations, consistency, dispersibility, pH, thickness, stability, skin irritation, efficacy against aging, and anti-inflammatory. **Results:** The anti-aging hydrogel made from *P. emblica* fruit nanosimplicia is a dark brown, uniform substance with a pH range of 5.26-6.28 and spreadability between 6.1-6.8. It remains stable after 4 weeks of storage at room temperature and is non-irritating to the skin. Results showed that the 6 % hydrogel preparation was the most effective in increasing moisture by 17.90 %, improving evenness by 15.83 %, reducing pore size by 15.30 %, diminishing pore spots by 22.67 %, and decreasing wrinkles by 28.06 %. Hydrogel concentrations of 2 %, 4 %, and 6 % showed inhibitory results of 0.7 %, 6.6 %, and 8.19 % respectively. **Conclusion:** *Phyllanthus emblica* nanosimplicia was successfully incorporated into a hydrogel formulation and remained stable for four weeks of storage. Hydrogel 6% exhibited superior anti-aging and anti-inflammatory properties.

Abbreviations: TEA, triethanolamine; BSA, bovine serum albumin; UPLC-ESI-QTOF-MS, Ultra Performance Liquid Chromatography coupled with Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometry; SEM, Scanning Electron Microscopy

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1. Introduction

The human body possesses multiple mechanisms for self-protection. The body's primary defense mechanism is a mechanical barrier provided by the skin [1]. The aging process is a process in which there is a decline of degeneration that causes the body to lose its functions and abilities, including causing the appearance of wrinkles and fine lines on the face or other body parts [2].

The predominant factor leading to this phenomenon is exposure to free radicals, which manifest as ultraviolet radiation. Free radicals consist of atoms or molecules possessing one or more unpaired electrons. The detrimental impact of free radicals on cellular health is well documented, as evidenced by their association with accelerated aging and a range of pathological conditions. Antioxidants, such as vitamins C and E, have been employed to sequester free radicals [3]. A hydrophilic system known as hydrogel primarily comprises 85–95% water and can undergo swelling. Hydrogels are characterized by their high water content and soft consistency [4].

Phyllanthus emblica L. fruit is a medicinal plant indigenous to Indonesia with therapeutic properties for treating diverse ailments. *Phyllanthus emblica*, commonly known as balakka fruit, is a botanical species frequently utilized by indigenous populations as a constituent in alternative medicine [5,6].

The fruit of *Phyllanthus emblica* L. is considered a rich source of vitamin C, and numerous investigations have been carried out to analyze its constituent composition [7]. Vitamin C in the fruit of *Phyllanthus emblica* exhibits antioxidant and anti-inflammatory properties, thereby conferring advantageous effects on skin health. Vitamin C exhibits potent antioxidant properties at a concentration of 0.02%, as evidenced by its IC_{50} value of 3.09 g/mL. The utilization of vitamin C's antioxidant activity in *Phyllanthus emblica* can serve as an effective anti-aging agent. Additionally, the anti-inflammatory properties inherent in the *Phyllanthus emblica* fruit can protect skin damaged by environmental factors such as sunlight, pollution, and toxins. Consequently, researchers are intrigued by the prospect of investigating the development and assessment of hydrogel formulations derived from *Phyllanthus emblica* L. fruit nanosimplicia and raising their anti-inflammatory efficacy [8, 9].

2. Materials and methods

2.1. Preparation of simplicia

The samples utilized were fruit from the *Phyllanthus emblica* L. plant collected from Badung Regency, Bali. Sample identification was conducted at the Medanense Herbarium, specifically at the Herbarium Laboratory of the Faculty of Mathematics and Natural Sciences of Universitas Sumatera Utara (5581/MEDA/2021). The fruit is rinsed with running water to remove debris, then dried at a temperature ranging from 40-50 °C. The dried fruit is pureed in a blender and stored in an airtight plastic jar to simplify it.

2.2. Nanosimplicia preparation

The simplicia powder was ground using a high-energy ball mill at PT. Indonesian Herbal Nanotech. The nanosimplicia characterization of *Phyllantus emblica* fruit was carried out using phytochemical analysis, particle size analyzer, Fourier transform infrared spectrometer, and scanning electron microscope.

2.3. Hydrogel preparation

The hydrogel formulation comprises a base of carbopol and Hydroxypropyl methylcellulose (HPMC), proposed by Harliatika and Noval (2021) [10]. The present study employed

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nanosimplicia derived from *P. emblica* fruit to formulate dosages with hydrogel. The simplicia *P. emblica* fruit nanosimplicia concentrations used in the formulations were 2 %, 4 %, and 6 %. In contrast, the basic formulation without nanosimplicia (*P. emblica*) was used as a blank preparation. Table 1 presents the formula design for hydrogel preparation using *Phyllanthus emblica* fruit nanosimplicia.

Table 1. *P. emblica* Fruit Nanosimplicia Hydrogel Formula Design

Ingredient	Formula (%)			
	1	**2**	**3**	**4**
Carbopol 940	0.375	0.375	0.375	0.375
HPMC	0.375	0.375	0.375	0.375
Glycerin	5	5	6	7
P. emblica Fruit Nanosimplicia	0	2	4	6
Methyl Paraben	0.18	0,18	0.18	0.18
Propyl Paraben	0.02	0,02	0.02	0.02
TEA	3 drops	3 drops	3 drops	3 drops
Aquadest *ad*	100 g	100 g	100 g	100 g

The hydrogel base consisting of carbopol and HPMC was subjected to a hot aquadest spreading process with a volume of 37.5 mL. The mixture was then allowed to rest for 24 hours. The base hydrogel was subjected to continuous stirring. A single droplet of triethanolamine (TEA) was introduced into a carbopol matrix and agitated until a uniform mixture was achieved. The two hydrogel bases were blended until a homogeneous, transparent hydrogel base was obtained. The partial dissolution of glycerin was observed in the presence of methylparaben and propylparaben. The process involved the addition of nanosimplicia and glycerin, followed by stirring until a state of homogeneity was achieved. The homogenization process involved the addition of a mixture of nanosimplicia into the hydrogel base, followed by grinding until a uniform mixture was achieved. A homogeneous hydrogel mass weighing 100 grams was obtained by adding aquades and TEA, followed by crushing.

2.4. Evaluation of physical quality of preparations

The physical characteristics of *P. emblica* fruit nanosimplicia hydrogel were assessed through various parameters such as organoleptic examination, homogenity, pH, viscosity, spreadability, and preparation stability.

2.5. Preparation of stability test

Concerning temperature variations, a cycling test assessed the preparation's physical stability over a specified storage duration. The experiment involved six cycles of the cycling test. The hydrogel preparation underwent a temperature cycling process, where it was initially stored at approximately 4 °C for 24 hours and then transferred to a temperature of roughly 40 °C. Each complete sequence of this process was considered a single cycle [11].

The stability of the preparation was tested by observing the organoleptic test, pH test, and viscosity test during the storage process at room temperature (20-25 °C); the changes were observed every seven days for 28 days. Observations were made on days 0, 7, 14, 21, and 28. Each preparation was put into a plastic pot [11].

2.6. Irritation test on volunteers

The irritation test is conducted to ascertain the potential of the preparation to irritate. The

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cycling test assesses the physical stability of the preparation during temperature fluctuations throughout a set storage time. The experiment involved conducting six cycles of the cycling test. The hydrogel formulation was subjected to a refrigeration temperature of approximately 4 °C for 24 hours, followed by a transfer to a temperature of roughly 40°C, considered a single cycle. The formulation selected for the irritation assessment had a maximum concentration of 6 %.

The method used technique involved using the use test, whereby the hydrogel formulation was administered onto the cutaneous region situated posterior to the auricle of the participant and subsequently allowed to remain in situ for 24 hours. Observe the chemical reaction that takes place. The manifestation of a positive irritation reaction is typified by erythema, pruritus, and edema in the targeted region [12].

2.7. Anti-aging Activity Test

The present study evaluated anti-aging activity by recruiting 12 participants into four groups.

A. Group I: 3 volunteers for a hydrogel formula without nanosimplicia

B. Group II: 3 volunteers for a hydrogel formula with a nanosimplicia 2 %

C. Group III: 3 volunteers for a hydrogel formula with a nanosimplicia 4 %

D. Group IV: 3 volunteers for a hydrogel formula with a nanosimplicia 6 %

Prior to the treatment, all volunteers underwent facial skin analysis using the Aramo-SG brand skin analyzer from the Aram Huvis company. The purpose of the initial analysis is to assess the initial skin condition based on predetermined measurement parameters, including:

1. Moisture: The water content was measured using the moisture checker tool in the Skin Analyzer device. To accomplish this, press the power button and affix it to the skin's surface. The number displayed on the device is the percentage of water content in the skin being measured [12].

2. Evenness: Skin smoothness was measured with a skin analyzer device at a 60x magnification lens and using a blue sensor light (normal). The camera is positioned on the skin's surface for measurement, followed by pressing the capture button, and the results in the form of numbers obtained will appear on the computer screen [12].

3. Pores: Pore size measurements on the skin will automatically appear when measuring skin smoothness. The image photographed on the skin smoothness measurement will also appear in the box section of the skin pores. The results in numbers will automatically appear on the computer screen [12].

4. Spots: The number of stains was measured using a skin analyzer device at a 60x magnification lens and an orange sensor lamp (polarized). The camera is placed on the skin's surface to be measured, then the button captures, and automatically, the results in the form of numbers and determination of the number of stains obtained will appear on the computer screen [12].

5. Wrinkles: Wrinkles measurements were carried out using a skin analyzer device with a 10x magnification lens and a blue sensor light (normal). The camera is placed on the skin's surface, which is measured, then press the capture button to take a photo, and automatically, the results in the form of numbers and skin conditions obtained will appear on the computer screen [12].

Following the baseline skin condition assessment, the intervention commenced with the application of hydrogel, which was uniformly

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distributed across the targeted region according to a predetermined grouping. The hydrogel was administered twice daily, in the morning and evening, over four weeks. Skin condition alterations were assessed weekly over four weeks using a skin analyzer [12].

2.8. Anti-inflammatory activity measurement by in vitro

The concentration of the test and positive control solutions was up to 50 mL each. 0.2 % BSA solution was added till the volume reached 5 mL. The mixture will result in 20 µg/mL concentration for each hydrogel dosage and solutions containing diclofenac sodium at concentrations (5, 10, 20, and 40 µg/mL). The sample was incubated at 25 °C for 30 minutes and then heated at 72 °C for 5 minutes. Subsequently, it was allowed to sit for 25 minutes at 23 °C. Following cooling, the solution was mixed vigorously in a vortex, and absorbance readings were obtained using UV-visible spectrophotometry at a wavelength of 660 nm. The anti-inflammatory activity test was conducted three times in triplicate [13, 14].

The percentage of protein denaturation inhibition was measured using the following formula:

$$\%inhibition \frac{absorbance\ of\ negative\ control - absorbance\ of\ test\ solution}{absorbance\ of\ negative\ control}\ x\ 100\%$$

Compounds that inhibit protein denaturation greater than 20 % have anti-inflammatory properties and can be used as reference values for drug development.

3. Results

3.1. Nanosimplicia

The measurement results indicate that the simplicia of Phyllanthus emblica fruit falls within the nanoparticle size range. The result of SEM which can be seen in Figure 1 show an irregular surface shape, uneven surface texture, and the formation of acute and obtuse corners with extremely small particle sizes (< 300 µm). Large particles up to 1000 µm are present. Nanosimplicia are particles ranging from 1 to 1000 nm, falling under the nanoparticle size category.

The phytochemical screening test was carried out to determine the class of chemical compounds contained in Phyllanthus emblica fruit nanosimplicia. The results of phytochemical screening can be seen in Table 2 below.

Table 2. Phytochemical screening of *Phyllanthus emblica* fruit nanosimplicia

No	Group	Nanosimplicia	Keterangan
1	Alkaloids	+	Bouchardat's reagent: blackish precipitate Dragendorff's reagent: orange yellow color
2	Flavonoids	+	Amyl alcohol layer: yellow color
3	Tannin	+	FeCl3 reagent: blue color
4	Saponins	+	Foam does not disappear with additions
5	Steroids/triterpenoids	+	HCl 2N
6	Glycosides	+	LB reagent: green

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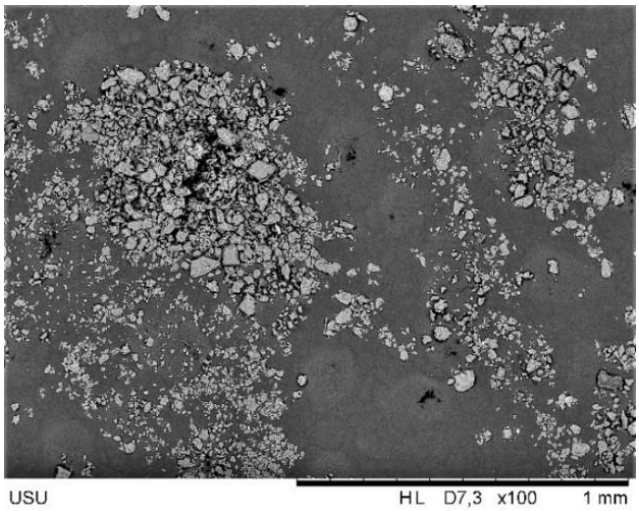

Fig. 1. Morphological results of nanosimplicia using SEM

Based on Figure 2, it can be seen that the wave number 3317 cm−1 is the frequency at which the −OH functional group stretches, 2954 cm−1 is the frequency at which the −C−H functional group stretches. The stretching frequencies of the −C=O functional group, the C-O (carbonyl) functional group, and the −C− O−C functional group are represented by the wave numbers 1724 cm−1, 1614 cm−1, and 1340 cm−1, respectively.

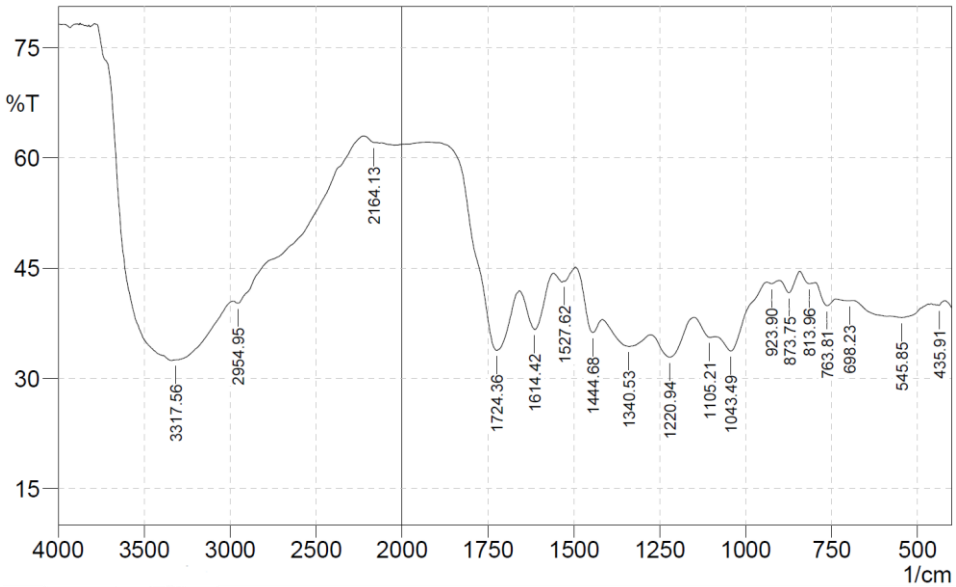

Fig. 2. FTIR spectra of nanosimplicia

3.2. Results of organoleptic observations of hydrogel preparations

The resulting hydrogel preparation exhibited a dark brown color and a distinctive aroma. No significant alterations in color, odor, or consistency were observed through organoleptic evaluations of hydrogels during a 4-week storage period at ambient temperature (20–25 °C).

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3.3. Results of observation of homogenity of hydrogel preparations

The observation of homogenity showed that all hydrogel preparations were homogeneous, and there were no coarse grains.

3.4. Results of hydrogel preparation pH measurement

The pH of the hydrogel using a pH meter after manufacture and at 0, 1, 2, 3, and 4 weeks during storage. The pH of the obtained nanosimplicia was determined to be 5.7. The pH values of each formula ranged from 5.26-6.28. It is noteworthy that cosmetic products must conform to the physiological pH range of the skin, which is between 4.5 and 6.5 (Table 3) [15].

Table 3. Results of pH measurement of hydrogel preparations from nanosimplicia

Formula	Observation Time (Day)				
	0	7	14	21	28
F0	5.26	5.26	5.26	5.26	5.27
F1	5.88	5.88	5.88	5.87	5.87
F2	5.89	5.89	5.89	5.89	5,88
F3	6.26	6.26	6.26	6.28	6.28

3.5. Result of Viscosity Measurement Hydrogel Preparation

The hydrogel formulations of *P. emblica* fruit nanosimplicia with different concentrations had viscosity values ranging from 50 dPa.S to 400 dPa.S or 5000 cP to 40000 cP, which were considered favorable (Table 4) [10].

Table 4. Viscosity measurement results of hydrogel preparations

Formula	Viscosity (Cp) Duration (Day)				
	0	7	14	21	28
F0	8974	8974	8974	8973	8973
F1	8167	8167	8167	8165	8164
F2	9081	9081	9081	9081	9081
F3	9844	9844	9844	9844	9844

3.6. Result of spreadability measurement hydrogel preparation

Results indicate that the hydrogel preparations exhibit good spreadability, as evidenced by the distribution falling within the standard spreading range and a good hydrogel dispersion range (Table 5).

Table 5. Spreadability measurement results of hydrogel preparation

Formula	Observation result (Diameter)			Spreadability
	50 g	100 g	150 g	
F0	6.1	6.5	6.9	good
F1	6.0	6.5	6.8	good
F2	5.5	5.8	6.2	good
F3	5.4	5.6	6.0	good

3.7. Result of cycling test

Results of the hydrogel preparation did not change the physical form after the cycling test, with the preparation being brown, with a distinctive odor, slightly viscous consistency, and no phase separation (precipitation). The results showed that the hydrogel preparation was resistant to temperature changes (Table 6).

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Table 6. Cycling test results for hydrogel preparations

Formula	Cycling test			
	Color	Odor	Consistency	Phase Separation (Precipitation)
F0	T	NS	ST	-
F1	B	SS	ST	-
F2	B	SS	ST	-
F3	B	SS	ST	-

T: Transparent , B: Brown, NS: No Smell, SS: Specific Smell, ST: Slightly Thick, -: No precipitation

3.8. Result of hydrogel preparation stability (Table 7)

Table 7. Observation results of hydrogel preparation stability

Parameter	Formula	Observation Duration (Day)				
		0	7	14	21	28
Color	F0	T	T	T	T	T
	F1	B	B	B	B	B
	F2	B	B	B	B	B
	F3	B	B	B	B	B
Odor	F0	NS	NS	NS	NS	NS
	F1	SS	SS	SS	SS	SS
	F2	SS	SS	SS	SS	SS
	F3	SS	SS	SS	SS	SS
Consistency	F0	ST	ST	ST	ST	ST
	F1	ST	ST	ST	ST	ST
	F2	ST	ST	ST	ST	ST
	F3	ST	ST	ST	ST	ST

F0, F1, F2, and F3 stored at room color, smell, and consistency unchanged since the beginning of the observation up to 4 weeks. Each hydrogel formula's preparation meets the organoleptic, pH, and viscosity evaluation requirements at room temperature.

3.9. Irritation test results on volunteers

The findings of an irritation test conducted on a sample of 12 participants indicated that all individuals exhibited negative responses to the parameters of irritation (Table 8 & Fig. 3).

Table 8. Irritation test results on volunteers

Observation	Volunteer											
Parameter	1	2	3	4	5	6	7	8	9	10	11	12
Redness	-	-	-	-	-	-	-	-	-	-	-	-
Itchiness	-	-	-	-	-	-	-	-	-	-	-	-
Swollen	-	-	-	-	-	-	-	-	-	-	-	-

- : no reaction

Fig. 3. Irritation test on volunteers

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3.10. Anti-aging Effectiveness Test Results

3.10.1. Moisture

The experimental use of nanosimplicia hydrogel yielded noteworthy outcomes, specifically with formulations F0, F1, F2, and F3. The data indicated that F3 exhibited the highest increase in water content on the participants' skin, with an average percentage increase of 17.9 %. In contrast, the blank formula demonstrated a minor increase, with an average percentage increase in water content of 4.51 % (Table 9).

Table 9. Data on the results of measurement of moisture on the Skin of volunteers

Formula	Volunteers	Moisture					Moisture increased (%)
		Before	Duration of usage (weeks)				
			1	2	3	4	
F0	1	31	31	31	31	32	3.22
	2	30	30	31	31	32	6.66
	3	28	28	28	28	29	3.57
	Mean	29.66	29.66	30	30	31	4.51
F1	1	30	31	32	32	33	10
	2	31	31	32	32	34	9.67
	3	35	36	36	37	39	11.42
	Mean	32	32.66	33.33	33.66	35.33	10.40
F2	1	28	29	31	32	33	17.85
	2	36	36	37	38	40	11.11
	3	34	35	36	38	39	14.70
	Mean	32.66	33.33	34.66	36	37.33	14.29
F3	1	34	35	37	38	40	17.64
	2	29	29	31	32	34	17.24
	3	32	35	35	36	38	18.75
	Mean	31.66	33	34.33	35.33	37.33	17.90

3.10.2. Pore

Volunteers experienced the highest pore reduction in F3 with an average percent pore reduction of 15.30 %, followed by F2, F1, and F0 with a pore reduction of 14.12 %, 12.18 %, and 4.24 % (Table 10).

Table 10. Data on Pore Size Measurement Results on Volunteer Skin

Formula	Volunteer	Pore size					Pore shrinkage (%)
		Before	Duration of usage (week)				
			1	2	3	4	
F0	1	29	29	29	28	28	3.44
	2	32	32	31	31	30	6.25
	3	33	33	32	32	32	3.03
	Mean	31.33	31.33	30.66	30.33	30	4.24
F1	1	30	30	29	27	27	10
	2	27	26	25	24	24	11.11
	3	25	24	23	23	21	16
	Mean	27.33	26.66	25.73	24.66	24	12.18
F2	1	26	26	24	22	21	19.23
	2	30	30	29	28	26	13.33
	3	36	35	35	33	32	11.11
	Mean	30.66	30.33	29.33	27.66	26.33	14.12
F3	1	38	37	37	35	33	13,15
	2	33	31	31	30	29	12,12
	3	27	25	24	23	21	22,22
	Mean	32.66	31	30.66	29.33	27.66	15.30

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3.10.3. Evenness

Volunteers experienced the highest increase in smoothness at F3 with an average percent increase in fineness of 15.83 %. F1 experienced an increase of 10.19 %, F2 12.14 %, and the blank formula experienced a slight increase with an average percent increase in fineness of 4.18 % (Table 11).

Table 11. Data on the Results of Evenness Measurements on the Skin of Volunteers

Formula	Volunteer	Evenness					Evenness increased (%)
		Before	Duration of usage (week)				
			1	2	3	4	
F0	1	31	31	31	30	30	3.22
	2	29	29	29	28	28	3.44
	3	36	36	36	35	34	5.55
	Mean	32	32	32	31	30.66	4.18
F1	1	28	28	26	25	25	10.71
	2	37	37	36	35	33	10.81
	3	33	32	32	30	30	9.09
	Mean	32.66	32.22	28	30	29.33	10.19
F2	1	38	38	36	35	34	10.52
	2	35	35	34	31	30	14,28
	3	34	34	33	31	30	11.67
	Mean	35.66	35.66	34.33	32.33	31.33	12.14
F3	1	39	39	37	36	34	12.82
	2	30	29	27	26	25	16.66
	3	32	31	30	28	26	18.75
	Mean	33.66	33	31.33	30	28.33	15.83

3.10.4. Spot

Using hydrogel nanosimplicia with the formula F0, F1, F2, and F3 showed that the number of blemishes on the volunteer's skin decreased. The highest number of stains was in F3, with an average percent decrease of 22.67 %. followed by F2, F1 and F0 with a decrease of 15.84 %, 10.34 % and 8.69 % (Table 12).

Table 12. Data on the results of measuring the number of spots on the skin of volunteers

Formula	Volunteer	Number of spots					The number of spot decreased (%)
		Before	Duration of usage (week)				
			1	2	3	4	
F0	1	24	24	24	24	23	4.16
	2	22	22	22	21	20	10.00
	3	33	33	33	32	32	3.03
	Mean	23	26.33	26.33	25.66	25	8.69
F1	1	32	32	30	29	29	9.37
	2	23	22	22	20	19	17.39
	3	32	32	31	30	30	6.25
	Mean	29	28.66	21.66	26.33	26	10.34
F2	1	27	27	26	25	23	18.51
	2	29	28	27	25	25	13.79
	3	26	25	22	21	21	19.23
	Mean	27.33	26.66	25	23.66	23	15.84
F3	1	36	36	34	31	30	16.66
	2	35	34	31	30	29	17.14
	3	26	25	22	21	19	26.32
	Mean	32.33	31.66	29	27.33	25	22.67

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3.10.5. Wrinkle

F0, F1, F2, and F3 formulations, decreased the wrinkles on the skin of the participants in the study. The F3 group exhibited the most significant reduction in percentage, with an average of 28.06 %, while F2, F1, and F0 experienced a decrease of 17.09 %, 12.84 %, and 1.47 %, respectively. The previous observation indicates a direct correlation between the concentration of nanosimplicia derived from *Phyllanthus emblica* L. fruit and the extent of reduction in wrinkle formation (Table 13).

Table 13. Data on the measurement of wrinkles on the volunteers' skin

Formula	Volunteer	Wrinkle					Wrinkle healed (%)
		Before	Duration of usage (week)				
			1	2	3	4	
F0	1	21	21	21	21	21	0
	2	29	29	28	28	28	3.44
	3	19	19	19	19	19	0.00
	Mean	23	23	22.66	22.66	22.66	1.47
F1	1	26	26	25	24	23	11.53
	2	24	23	23	21	20	16.66
	3	28	27	27	26	25	10.71
	Mean	26	25.33	25	23.66	22.66	12.84
F2	1	27	25	25	24	23	18.51
	2	24	24	22	20	19	20.83
	3	28	24	24	23	21	16.00
	Mean	25.33	24.33	23.66	22.33	21	17.09
F3	1	28	27	25	22	20	28.57
	2	26	25	22	21	19	26.92
	3	28	27	24	23	20	28.57
	Mean	27.33	26.33	22.66	22	19.66	28.06

3.11. Anti-inflammatory Activity Results Against Inhibition Denaturation of Proteins In Vitro

The anti-inflammatory activity results against the positive control at concentrations of 5 ppm, 10 ppm, 20 ppm, and 40 ppm, as well as the test solution.

Based on the percentage inhibition data, the negative control did not exhibit any anti-inflammatory action. The percentage of inhibition in the positive control increased as the concentration increased, which was also applied to the hydrogel test solution. The results of the 6 % hydrogel showed the greatest inhibition of 8.19 % (Table 14).

Table 14. Anti-inflammation Activity

Concentration	Absorbance	Percent inhibition (%)
Negative Control	2,380	0.00
Positive Control 5 mg/L	2.149	9.70
Positive Control 10 mg/L	2.092	12.10
Positive Control 20 mg/L	2.081	12.56
Positive Control 40 mg/L	2.063	13.31
Hydrogel 2% ten mg/L	2.363	0.7
Hydrogel 4% ten mg/L	2.221	6.6
Hydrogel 6% ten mg/L	2.185	8.19

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4. Discussion

The Simplicia that was sent to PT. Nanotech Herbal Indonesia will be used in a nano size (731 ± 168 nm) and functional groups indicate polyphenolic compounds, pectin, and vitamin C in *P. emblica* fruit simplicia nanosimplicia. Based on Sabir et al. 2015 the total amount of phenol and vitamin C in Simplia was 13.4 % and 5.4 % respectively [16]. However, the preparation's pH remained within the skin's pH range, typically between 4.5 and 6.5 [15]. The pH test is a crucial step in assessing the acidity level of preparations to ensure that they do not induce skin irritation or dryness. The decrease in pH in the hydrogel can be affected by light and humidity. In a gel base that uses a carbopol base, a decrease in pH can occur due to the reaction between the carboxylate group on the carbopol with water so that more H_3O^+ (acid) is formed, making the gel base more acidic [15].

The viscosity test is a valuable tool for determining the viscosity of a preparation. The viscosity value indicates a liquid's resistance to flow at 20 ˚C. Viscosity describes the viscosity of a preparation related to dispersion. Preparations with a lower viscosity (waterier) produce a bigger spreading diameter because it is easier to flow. Besides, because of lower viscosity, the diameter of the spread becomes wider. Viscosity testing of hydrogel nanosuspensions was conducted using a viscometer. The decrease in viscosity can be due to the nature of carbopol, which is hygroscopic, so it can absorb moisture, which causes a stock to have decreased viscosity or be diluted [17].

Spreadability measurement was done to guarantee that the preparation could apply when pressure was applied so it would spread easily without causing pain to guarantee user convenience. An optimal hydrogel formulation typically falls within the 5-7 cm range. Preparations possessing a diameter of 50 mm were incorporated within the semi-rigid spread. In contrast, preparations with a diameter greater than 50 mm but less than 70 mm were included within the semi-fluid dispersion. This hidrogel has a good spreadability [18].

Stability assessments were conducted to determine if any alterations occurred in the formulation throughout its storage. The appearance of discoloration or appearance of color, odor, change or phase separation, syneresis, changes in consistency, gas formation, and other physical changes characterizes the physical instability of the preparation. The resulting preparation was stable because the nanosimplicia hydrogel preparation did not undergo physical changes [17, 18].

The outer layer irritation test was conducted to evaluate the potential adverse effects of cosmetics on the skin. The test involved the application of the cosmetic product on the underside of the arm or behind the ear, followed by a 24-hour observation period to assess the outcomes. An irritation test was performed on a stable nanosimplicia hydrogel with a concentration of 6 %. The parameters that were observed encompassed the presence of erythematous skin, pruritus, or edema. Therefore, it can be inferred that the nanosimplicia hydrogel was safe for use [17, 18].

The moisture checking results show increased water content on the facial skin of volunteers for four weeks of treatment by administering the hydrogel preparation of nanosimplicia with the use of the preparation every day, twice a day for four weeks regularly. The observation above suggests that an escalation in the concentration of nanosimplicia results in a proportional augmentation of skin moisture content. The observed rise in water content of the blank formula can be attributed to the presence of

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glycerin in the hydrogel base, which functions as a humectant and enhances skin hydration within the stratum corneum layer [19].

The higher the nanosimplicia concentration, the greater the pore size reduction. Vitamin C in the hydrogel nanosimplicia led to a decrease in pore size. Fruit, which can stimulate collagen production. The collagen produced can then shrink the pores on the skin [20]. The size of the pores was closely related to the skin's smoothness. The smaller the pores on the skin, the smoother the skin; the larger theores, the rougher the skin.

The higher the nanosimplicia concentration, the greater the resulting increase in skin smoothness. The manifestation of premature aging on the skin is often characterized by symptoms such as dryness and roughness. Frequent exposure of the skin to sunlight, particularly ultraviolet radiation, can result in the degradation of collagen and elastin within the skin layer. This, in turn, leads to the accumulation of dead cells in the stratum corneum, ultimately resulting in a rougher skin texture. Furthermore, the epidermis may exhibit a rough, lacklustre, and flaky texture due to impaired exfoliation of aged keratinocytes and subsequent replacement with new cells. The content contained in *Phyllanthus emblica* L. fruit, such as vitamin C, is important in smoothing the skin because vitamin C is an antioxidant that can counteract free radicals that cause skin damage. Vitamin C can increase skin elasticity (smoothness) by catalyzing hydroxyproline and hydroxylysine-producing enzymes that form collagen mass [20, 21].

The higher the nanosimplicia of *Phyllanthus emblica* L. fruit, the greater the decrease in the number of stains produced. Dark spots (hyperpigmentation) are a condition with an increase in the amount of melanin in the skin layer, which results in a darker skin color change. Vitamin C plays a role in converting the oxidized form of melanin to a lighter-colored reduced form of melanin and prevents the formation of melanin by inhibiting the formation of dopa into dopa quinone. Thus, the skin becomes brighter on normal skin and those with pigmentation disorders (hyperpigmentation) [19, 22].

The results of this investigation demonstrate that nano simplicia derived from *Phyllanthus emblica* L. fruit exhibit significant anti-aging properties. This aligns with existing literature confirming the anti-wrinkle effects of *Phyllanthus emblica* L. extract. Comprehensive phytochemical analysis using Ultra Performance Liquid Chromatography coupled with Electrospray Ionization and Quadrupole Time-of-Flight Mass Spectrometry (UPLC-ESI-QTOF-MS) has identified a robust presence of flavonols and phenolic acids in the fruit, including quercetin, myricetin, ellagic acid, gallic acid, and chlorogenic acid, as well as their respective glycosides, which are thought to contribute to its anti-inflammatory and skin rejuvenation activities [23, 24, 25].

The occurrence of wrinkles or wrinkles, a reduction in the thickness of the dermis by as much as 20 % in the elderly, is related to the loss of elastin and collagen fibers. Collagen and elastin are the main components of the dermis layer. The loss of these fibers adversely affects the moisture and tension of the skin, causing wrinkles or wrinkles. Anti-wrinkle or anti-wrinkle to eliminate the impact of UV rays and an anti-wrinkle/anti-wrinkle, and many cosmetics contain antioxidants, such as vitamin C. Antioxidants function to capture free radicals in the skin caused by UV rays and the population. Vitamin C works as an antioxidant, which prevents the oxidation of the skin's constituent fibers, namely collagen and elastin, at the

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expense of being oxidized by free radicals. In addition, vitamin C also functions as a co-factor (one of the factors that accelerates the formation of collagen because vitamin C also functions as an antioxidant that can renew dead skin cells into new ones through collagen formation). The synthesis of collagen and the outermost layer of the skin. Vitamin C stimulates and increases skin collagen production by increasing the ability to proliferate old dermis fibroblast cells [19].

Diverse etiological factors, including but not limited to prolonged exposure to ultraviolet radiation, the natural aging process, hormonal fluctuations during gestation, or the administration of certain pharmaceuticals, may precipitate cutaneous pathologies. The hydrogel, composed of nanosimplicia derived from the fruit of *Phyllanthus emblica* L, exhibits anti-inflammatory properties and may mitigate the primary contributors to the aging process [26, 27].

5. Conclusion

The hydrogel of *Phyllanthus emblica* L fruit nanosimplicia showed an improved skin condition after four weeks of treatment. The Nanosimplicia hydrogel can provide an anti-aging effect, with the *Phyllanthus emblica* fruit nanosimplicia hydrogel with a concentration of 6 % showing the highest effectiveness compared to 2 % and 4 % concentrations, with an increasing water content of 17.90 %, pore reduction by 15.30 %, skin evenness increased by 15.83 %, spot reduction by 22.67 %, and wrinkles decreased by 28.06 % after four weeks of treatment. The anti-inflammatory activity showed the greatest inhibition of 8.19% among the formula.

Author contributions

All authours searched for the articles, and J wrote the first draft. M and S designed the study and contributed to the writing process and analysis. SH was the study supervisor, contributed to all aspect of the study, and provided the final manuscript. J contributed to the study process. All authors read and approved the paper.

Conflict of interest

The authors declare no conflict of interest.

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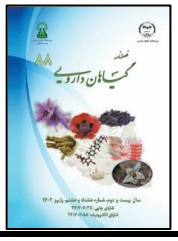

مقاله تحقیقاتی

فعالیت ضد التهابی و ضد پیری هیدروژل با ماده فعال *Phyllantus emblica* L. nanosimplicia میوه

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اطلاعات مقاله

گل‌واژگان:

فرمولاسیون

هیدروژل

نانو ساده‌سازی

Phyllantus emblica L.

ضد پیری

چکیده

مقدمه: **گیاه** *Phyllantus emblica* با دارا بودن سطوح بسیار بالایی از ویتامین C به عنوان یک آنتی‌اکسیدان، دارای خاصیت ضد پیری زودرس و ضد التهاب است. این گیاه به دلیل داشتن قدرت پخش خوب روی پوست به صورت هیدروژل فرموله شده است. **هدف:** این تحقیق با هدف ایجاد فرمول هیدروژل از میوه گیاه *Phyllantus emblica* L. به عنوان یک ضدالتهاب و ضد پیری انجام شده است. **روش بررسی:** نانو ذرات با استفاده از آسیاب گلوله‌ای با انرژی بالا ساخته می‌شود و از طریق آنالیزهای فیتوشیمیایی، اندازه ذره ای، طیف‌سنجی فروسرخ تبدیل فوریه و میکروسکوپ الکترونی روبشی مشخصات آنها تعیین میگردد. هیدروژل‌ها با غلظت‌های (۲، ۴، ۶ درصد) فرموله می‌شوند. ارزیابی هیدروژل‌ها شامل ارزیابی‌های ارگانولپتیک، قوام، پراکندگی، pH، ضخامت، پایداری، تحریک پوستی، اثربخشی در برابر پیری و ضد التهاب بود. **نتایج:** هیدروژل ضد پیری ساخته شده از میوه *P. emblica* یک ماده قهوه‌ای تیره و یکنواخت با درجه اسیدی ۵/۲۶-۶/۲۸ و قابلیت پخش بین ۶/۱-۶/۸ بود. این هیدروژل پس از ۴ هفته نگهداری در دمای اتاق ÷ایدار ماند و برای پوست تحریک پذیر نبود. نتایج این مطالعه نشان داد که هیدروژل ۶ درصد در افزایش رطوبت به میزان ۱۷/۹۰ درصد، بهبود یکنواختی به میزان ۱۵/۸۳ درصد، کاهش اندازه منافذ به میزان ۱۵/۳۰ درصد، کاهش لکه‌های منافذ به میزان ۲۲/۶۷ درصد و کاهش چین و چروک‌ها به میزان ۲۸/۰۶ درصد مؤثر بوده است. هیدروژل های ۲، ۴، و ۶ درصد به ترتیب ۰/۷، ۶/۶ و ۸/۱۹ درصد بازدارندگی را نشان داد. **نتیجه‌گیری:** هیدروژل حاصل از نانوذرات میوه گیاه *Phyllanthus emblica*a پس از گذشت چهار هفته از ذخیره‌سازی پایدار ماند و هیدروژل ۶ درصد خواص ضد پیری و ضد التهابی بیشتری در مقایسه با هیدروژل های ۲ و ۴ درصد نشان داد.

مخففها: TEA، تری اتانول آمین؛ BSA، آلبومین سرم گاوی؛ UPLC-ESI-QTOF-MS، کروماتوگرافی مایع با عملکرد فوق العاده همراه با یونیزاسیون الکترواسپری و طیف سنجی جرمی زمان پرواز چهار قطبی؛ SEM، میکروسکوپ الکترونی روبشی

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