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Research Article

Development and Evaluation of Physicochemical Quality of Cantigi Leaf Extract-Based Eye Cream

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Abstract

Cantigi leaves are known to contain potent antioxidants. This study aimed to develop an eye cream formulation incorporating Cantigi leaf extract and gelatin nanoparticles, ensuring optimal physical quality, stability, and antioxidant activity. Cantigi leaves were ground and extracted using 70% ethanol, then concentrated using a rotary evaporator. Antioxidant activity was tested, followed by the preparation of gelatin nanoparticles using the nanoprecipitation method. These nanoparticles were freeze-dried and subjected to further antioxidant testing. Three formulations of the Blank, F1 containing an extract and F2 containing nanoparticles, were developed into an eye cream, which underwent antioxidant activity and stability assessments over four weeks, including organoleptic evaluation, pH measurement, homogeneity, viscosity, cream type analysis, and spreadability tests. The results showed that the IC_{50} value for Cantigi leaf extract was 17.40 ppm, while for Cantigi leaf extract gelatin nanoparticles, it was 33.58 ppm. F1 exhibited a light brown color, with viscosity values of 127857 cPs. Its flow properties were classified as thixotropic plastic, and it showed an IC_{50} value of 48.78 ppm, indicating its antioxidant capacity. F2 displayed a light orange color, with viscosity values of 125086 cPs. Similar to F1, its flow properties were also thixotropic plastic. The IC_{50} value was recorded at 61.87 ppm, reflecting its antioxidant potential. The irritation test demonstrated that both F1 and F2 did not cause skin irritation, based on rabbit skin irritation scores recorded at 1 hour, 24 hours, 48 hours, and 72 hours. The findings confirmed that Cantigi leaf extract and Cantigi leaf extract gelatin nanoparticles exhibit antioxidant and anti-inflammatory activity. Furthermore, they can be effectively formulated into a stable eye cream that does not cause skin irritation.

Keywords: Cantigi Leaves; Cantigi Leaf Extract; Cantigi Leaf Extract-Loaded Gelatin Nanoparticles; Eye Cream; DPPH; IC_{50}

Introduction

In recent years, awareness of skin health has expanded beyond the entire face to focus on specific areas, such as the lips, nose, chin, and eyes. One common issue affecting these areas is hyperpigmentation, which can present as localized pigmentation or spots. Periorbital hyperpigmentation, commonly known as dark circles, is a prevalent dermatological concern. According to an Indian study, 47.50% of individuals aged 16 to 25 years were found to have periorbital hyperpigmentation. The study also noted that the condition was more common in women, with 81% of affected individuals being female. Periorbital hyperpigmentation can result from various factors, including genetics, excessive pigmentation, post-inflammatory hyperpigmentation, vascular changes, and skin laxity due to aging. Treatment options range from topical depigmenting agents (such as hydroquinone, kojic acid, and azelaic acid) to physical therapies like chemical peels, laser treatments, and surgical corrections [1].

The skin beneath the eyes, known as the periorbital region, is particularly susceptible to dark circles (eye bags) due to its low-fat content, making it prone to dryness and reduced elasticity. These changes are often linked to premature aging. One effective solution for treating dark circles is the application of eye cream formulations [2,3].

Given the concerns surrounding premature aging, eye cream formulations can be enriched with active antioxidant substances to neutralize free radicals—a major contributor to skin aging. A natural ingredient with notable antioxidant properties is Cantigi leaves (Vaccinium varingiaefolium (Blume) Miq.). Previous research has demonstrated that Cantigi leaves exhibit an antioxidant activity of 18.80 ppm [4].

To enhance the antioxidant potential of Cantigi leaves, they can be formulated into nanoparticles. Due to their small size, nanoparticles can penetrate cellular spaces more effectively, improving bioavailability. Gelatin is used as a nanoparticle carrier because of its biodegradable, non-toxic properties, its ease of chemical modification, and its affordability [5].

Based on the above information, this study aimed to develop an eye cream incorporating Cantigi leaf extract and gelatin nanoparticles to enhance physical quality, stability, and antioxidant efficacy [6].

Materials and Methods

Materials

Cantigi leaves (Vaccinium varingiaefolium (Blume) Miq.) from White Crater, Ciwidey, Bandung; stearic acid, cetyl alcohol, paraffin liquidum, triethanolamine, phenoxyethanol, BHT, glycerin, propylene glycol, and distilled water are pharmaceutical grades.

Methods

Plant identification

The Identification of Cantigi leaves was conducted at the Herbarium Depokensis (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia [7].

Preparation of simplicia powder

Cantigi leaves were cleaned and dried in an oven at a low temperature (30-40˚C). The dried simplicia was then ground and sieved using sieve number 4, followed by another sieving using sieve number 18 [8].

Preparation of cantigi leaf extract

The extraction process was performed using the maceration method. A 500-gram sample of simple powder was weighed, and 5 liters of 70% ethanol were added. The mixture was macerated for 6 hours using a kinetic macerator, then concentrated with a rotary evaporator at 45˚C to obtain a thick ethanol extract (70%). The final extract was weighed, and the native DER and yield were calculated [8].

Characterization of cantigi leaf extract

Characterization included phytochemical screening, organoleptic evaluation, pH measurement, water content determination [8], and antioxidant activity assessment using the DPPH method [9].

Gelatin nanoparticle synthesis

Material	Quantity
Gelatin (mg)	5
Pure water (mL)	1
Cantigi leaf extract (mg)	3
DMSO (mL)	0.2
Ethanol 96% (mL)	10
Glutaraldehyde (mL)	0.5
Poloxamer 188 (mg)	700
DMSO (mL)	0.2
Pure water (mL)	1
Cantigi leaf extract (mg)	3

Table 1: Nanoparticle formulation containing Cantigi leaf extract.

First, 5 mg of gelatin was dissolved in 1 mL of pure water at 50°C. Separately, Cantigi leaf extract was prepared by dissolving it in 0.2 mL of DMSO and 0.2 mL of 96% ethanol, then stirred until fully dissolved. Next, 700 mg of poloxamer 188 was mixed with 10 mL of 96% ethanol and stirred using a magnetic stirrer until fully dissolved. The gelatin-extract mixture was then gradually added into the poloxamer 188 solution, drop by drop at 700 rpm. After 15 minutes of continuous stirring, 0.5 mL of 2% glutaraldehyde was added, and the solution was stirred for 12 hours using a magnetic stirrer. The resulting nanoparticles were centrifuged and purified by washing with pure water three times, each lasting 20 minutes. Finally, the purified nanoparticles were freeze-dried for storage and further analysis [10].

Characterization of Gelatin Nanoparticles

Particle Size and Polydispersity Index

The particle size and polydispersity index were analyzed using a Particle Size Analyzer [8].

Zeta potential

The zeta potential was measured using a Zetasizer, and the examination was conducted three times [8].

Entrapment efficiency

The entrapment efficiency (EE%) of a drug in gelatin nanoparticles (GNPs) is calculated using the following formula:[8]

EE% = [(Total drug concentration - Free drug concentration)/ Total drug concentration]*100

Nanoparticle Morphology

The morphology of the nanoparticles was determined using Scanning Electron Microscopy (SEM) [8].

FTIR Functional Group Determination

A sample weighing approximately ±2.0 mg was placed into a receptacle and analyzed using an FTIR spectrophotometer [11].

Antioxidant Activity Test of Nanoparticles Using the DPPH Method [8].

Preparation of DPPH Solution

A total of 4 mg of DPPH was carefully weighed and placed into a measuring flask, then dissolved in 25 mL of pro-analysis methanol. The prepared DPPH solution was stored in a dark glass bottle.

- Blank Solution Preparation
- A volume of 1500 µL of pro-analysis methanol (blank) was pipetted into a test tube, followed by the addition of 500 µL of DPPH solution. The mixture was shaken until homogeneous and incubated for 30 minutes at room temperature.
- Preparation of the Test Solution

A total of 10 mg of Cantigi leaf extract microcapsules was carefully weighed and dissolved in 10 mL of pro-analysis methanol (1000 ppm concentration) to prepare the stock solution. A series of solutions were then prepared with concentrations of 5, 10, 15, 20, and 25 µg/mL. Each concentration series was mixed with 1 mL of DPPH solution, and the final volume was adjusted to 5 mL using pro-analysis methanol. The solutions were shaken until homogeneous and incubated for 30 minutes at room temperature.

Calculation of inhibition percentage

The percentage of inhibition was determined using the following formula: % inhibition = [(Absorbance of Blank - Absorbance of Sample)/Absorbance of Blank] × 100%

Eye Cream formulations of extract and extract-loaded gelatin nanoparticles

The tools and materials required were prepared. The formulation components were separated into two phases: the water phase and the oil phase [12]. The oil phase, including stearic acid, cetyl alcohol, liquid paraffin, butylhydroxytoluene (BHT), and phenoxyethanol, was placed into a porcelain cup and heated in a water bath at 60-70°C until all ingredients melted completely. The water phase, consisting of triethanolamine, glycerin, and propylene glycol, was dissolved in water at a temperature of 60-70°C. The oil and water phases were combined in a heated mortar, stirred using a pestle at 70°C until a homogeneous cream base was formed. The mixture was then cooled to 40°C. The cantigi leaf extract or its nanopar-

Material	Blank	F1	F2
Cantigi leaf extract (g)	-	0.174	-
Cantigi leaf extract-loaded nanoparticles (g)	-	-	0.33
Stearic acid (g)	18	18	18
Cetyl alcohol (g)	0.5	0.5	0.5
Liquid paraffin (mL)	7	7	7
Propylene glycol (mL)	10	10	10
Glycerin (mL)	8	8	8
Phenoxyethanol (mL)	Qs	Qs	Qs
Butylhydroxytoluene (g)	Qs	Qs	Qs
Pure water	ad 100	ad 100	ad 100

Table 2: Eye Cream Formulations.

ticles were dissolved in a suitable solvent. The dissolved extract or nanoparticles were incorporated into the cream base using a pestle and mixed until fully homogeneous. The finished cream was transferred into appropriate packaging containers.

Evaluation of cantigi leaf extract eye cream [12]

Organoleptic examination

The organoleptic properties of the cream, including texture, odor, and color, were visually assessed. Testing was performed in triplicate for each formulation.

Cream type test

The type of cream was determined using a coloring technique. Three drops of methylene blue were added to three drops of cream and observed under a microscope. If the emulsion appeared uniformly colored, the cream was classified as type M/A.

Homogeneity test

The cream preparation was applied to a glass slide, which was then covered with another glass slide. The homogeneity of the cream was assessed visually.

Viscosity and flow properties test

The viscosity of the cream was measured using a Brookfield RV viscometer. The eye cream was placed in a glass cylinder container, and the spindle was lowered into the cream. The spindle was allowed to rotate, and the scale reading indicated by the red needle was recorded. The result was multiplied by the factor provided in the reference table to determine viscosity.

Viscosity was calculated using the formula

Viscosity (η) = (scale x multiflication factor) cPs

Forse (F) = (scale x Kv) dyne/cm^2

Spreadability test

A 0.5g sample of cream was placed in the center of an inverted petri dish. After resting for 1 minute, weights ranging from 50 g to 250g were sequentially applied every minute. The spread diameter was recorded, with the standard acceptable spread ranging between 5 cm and 7 cm. Testing was conducted in triplicate for each formula.

pH Test

The pH test was performed to ensure the safety of the cream and prevent skin irritation. A 1 g sample of cream was diluted with 10 mL of distilled water. The pH meter was calibrated using standard buffer solutions of pH 4 and pH 7. The electrode was rinsed with distilled water, dried with tissue, and immersed in the cream sample until a stable pH reading was obtained.

Antioxidant activity test

Preparation of Test Solution: A total of 10 mg of the capsule contents was carefully weighed and dissolved in 10 mL of pro-analysis methanol (1000 ppm concentration) to prepare the stock solution. A series of solutions with concentrations of 35, 40, 45, 50, and 55 µg/mL were then prepared. Each concentration series was mixed with 1 mL of DPPH solution, and the final volume was adjusted to 5 mL using pro-analysis methanol. The solutions were shaken until homogeneous and incubated for 30 minutes at room temperature [13].

Irritation Test [14,15]

Preparation of test animals

The test animals were healthy adult male or female albino rabbits weighing 2-2.5 kg. Before testing, the rabbits were acclimated to the experimental room for five days and housed individually in separate cages (one rabbit per cage). At least one day before testing, the fur was saved (10 × 15 cm^2 or 10% of the body surface), and the six positions were designated for sample application.

Treatment of test animals

The test preparation was applied to gauze as much as 0.5g for each area of 6 cm2 or 2x3 cm2. The application location was covered with gauze and plastered with a non-irritant material.

Three test animals underwent six sample applications each, with a four-hour exposure period. Residual samples were washed post-application using water or appropriate solvents. Skin irritation was observed at 1, 24, 48, and 72 hours and assessed based on the irritation score table, focusing on erythema and edema formation.

Evaluation of test results

The skin irritation score was assessed based on the severity of the wound and whether the condition was reversible. The primary irritation index for the test preparation was determined by analyzing all observations recorded throughout the test. This index was measured using the following formula:

Primary irritation index = (A-B)/C.

Where is A: the sum of the erythema and edema scores of all sample observation points at 24, 48, and 72 hours divided by the number of observations; B: the sum of the erythema and edema scores of all control observation points at 24, 48, and 72 hours divided by the number of observations; and C: Number of animals

The score classification of primary irritation index

- No irritation: 0 - 0.4
- Slight irritation: 0.5 - 1.9
- Moderate irritation: 2.0 - 4.9
- Severe irritation: 5.0 - 8.0

Results and Discussion

Results

Plant identification

Plant determination was conducted at the Herbarium Depokensis (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia. According to Letter No. 115/UN2.F3.11/PDP.02.00/2023, the leaves used were identified as true cantigi leaves, belonging to the species *Vaccinium varingiaefolium* (Blume) Miq. from the Ericaceae family.

Plant determination, also known as botanical identification, offers several important benefits, particularly in research, medicine, agriculture, and environmental conservation: Accurate identification, medicinal applications, ecological conservation, agricultural benefits, food safety, scientific research, commercial uses, and sustainability [16].

Preparation of simplicia powder

The fineness of the herbal powder was assessed using sieves. The results showed that 100% of the herbal powder passed through sieve number 4, while 24.35% passed through sieve number 18.

The fineness of the powder influences the efficiency of compound extraction during the extraction process. The fineness of the herbal powder met the standard of the Indonesian Herbal Pharma-

copoeia (FHI) [17]. "DER-native" in extraction seems to relate to Drug Extract Ratio (DER), which is a key factor in standardizing herbal extracts. DER represents the ratio between the amount of raw plant material used and the final extract obtained. A native extract refers to an extract that has not been modified with additional substances like carriers or preservatives, meaning it retains its original composition [18].

Preparation of cantigi leaf extract

Extraction was performed using the maceration method, where 502.34 g of simplicia powder were soaked in 5 liters of 70% ethanol. This process resulted in 156.9 g of thick extract, yielding 31.23%, with a DER-native value of 3.20. The DER-native value represents the amount of simplicia required to produce one gram of extract.

Characterization of cantigi leaf extract

Parameter	Description/Value
Organoleptic Properties:	
Smell	Distinctive and characteristic
Form	Thick extract
Color	Dark brown
pH	4.3 ± 0.005
Water Content (%)	8.21
Phytochemicals:	
Alkaloid	+
Saponins	+
Tannin	+
Phenolic	+
Flavonoid	+
Triterpenoid	+
Steroid	-
Glycosides	+
Metal Content:	
Lead (Pb)	Not detected
Cadmium (Cd)	Not detected
Antioxidant Activity:	
Reference standard (Vitamin C)	2.75 ± 0.040 ppm
Thick Extract of Cantigi Leaves	17.40 ± 0.99 ppm

Table 3: Characterization of Cantigi Leaf Extract.

The organoleptic evaluation confirmed that the thick extract of cantigi leaves had a dark brown color and a characteristic Cantigi odor. (Table 3) The pH test was conducted using buffer solutions of pH 4 and 7, yielding an average pH of 4.3. This result is attributed to the presence of flavonoid compounds in the extract, which were effectively extracted using 70% ethanol, as well as the presence of oleic acid in the cantigi leaf extract. (Table 3) The required water content for herbal extracts is ≤10% [17].

The water content of the cantigi leaf extract was found to be 8.21%, indicating compliance with the standard. The purpose of water content testing was to ensure the extract remains stable and resistant to microbial contamination, as water serves as a favorable medium for mold growth.(Table 3) Phytochemical screening of the cantigi leaf extract revealed the presence of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. Among these, phenolic compounds play a significant role as natural antioxidants [17]. (Table 3).

Metal content analysis was performed to ensure the extract's safety for use as a natural medicinal product. The results confirmed that lead (Pb) and cadmium (Cd) were not detected, indicating that the extract does not pose a risk of chronic poisoning [17]. (Table 3).

Antioxidant activity was assessed using a vitamin C standard, as vitamin C is a widely available synthetic antioxidant with strong antioxidant properties. The results demonstrated that cantigi leaf extract exhibits strong antioxidant activity when compared to the reference standard, suggesting its potential effectiveness as a natural antioxidant [8]. (Table 3)

Gelatin nanoparticle synthesis and characterization

Particle size distribution and polydispersity index

The Cantigi leaf extract gelatin nanoparticles were successfully prepared using a gelatin-based encapsulation method. The particle size of the Cantigi leaf extract nanoparticles was measured using a Particle Size Analyzer, yielding an average particle size of 235.8 nm. This size falls within the acceptable nanoparticle range of 10-10,000 nm [19]. The polydispersity index was recorded with an average value of 0.226, indicating that the nanoparticles were relatively homogeneous. A polydispersity index value close to 0 signifies high uniformity in nanoparticle dispersion [20].

Formula	Particle Size (nm)	Polydispersity Index
1	250.2	0.288
2	226.5	0.207
3	230.8	0.185
Mean ± SD	235.8 ± 12.62 nm	0.226 ± 0.05

Table 4: Particle size and polydispersity index.

Measurement	Zeta Potential (mV)
1	-8.85 mV
2	-8.41 mV
3	-8.34 mV
Mean ± SD	-8.53 ± 0.27 mV

Table 5: Zeta potential.

Gelatin nanoparticle morphology

The morphological analysis was performed using Scanning Electron Microscopy (SEM) at magnifications of 2500× and 5000×.

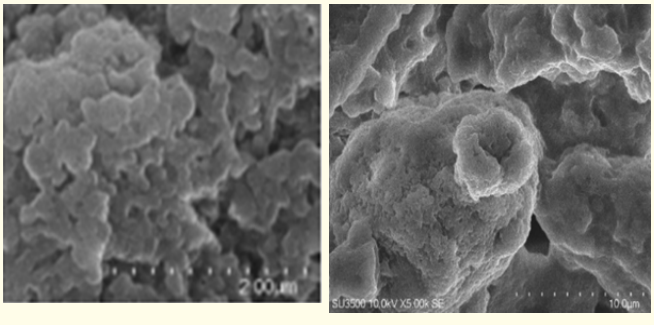

Figure 1: Gelatin nanoparticle morphology using a SEM.

The nanoparticles exhibited a relatively spherical shape, which is attributed to the presence of gelatin that forms a protective layer around the extract. The spherical morphology helps minimize particle-to-particle interactions, preventing aggregation [21].

Zeta potential measurement

A desirable zeta potential value is generally between (+/-) 30 mV. The Cantigi leaf extract nanoparticles exhibited an average zeta potential of -8.53 mV, indicating a negative charge. This negative charge can be attributed to poloxamer 188, a surfactant with a negative charge, which was present in significant concentration in the nanoparticle formulation. Zeta potential is a measure of the electrostatic potential difference between the surface of a charged particle and the surrounding fluid, specifically at the slipping plane. It quantifies the magnitude of electrostatic repulsion or attraction between particles in a suspension, influencing their stability [22].

Determination of entrapment efficiency

Entrapment efficiency of nanoparticles refers to the percentage of a drug or compound that is successfully encapsulated within the nanoparticles. It's a crucial parameter in nanoparticle-based drug delivery systems, influencing drug release, efficacy, and toxicity. A high entrapment efficiency means a larger portion of the drug is enclosed within the nanoparticles, potentially leading to better targeted delivery and reduced drug loss. Based on the calculated entrapment efficiency, the encapsulated compound successfully adsorbed within the nanoparticle system, achieving an entrapment efficiency of 52.69% [23].

Concentration (mg/mL)	Absorbance
100	0.7904
80	0.6523
60	0.4625
40	0.3572
20	0.2223
Y = 0.0676+0.0072x	R^2 = 0.9926
%EE = 52.69 ± 0.05 %	

Table 6: Concentration vs absorbances curve for entrapment efficiency measurement.

FTIR functional group determination

Functional group identification was performed using FTIR, with samples prepared in KBr pellets. The peak wave numbers indicated the presence of specific functional groups. The results revealed a sharp band at 3280.39 cm^{-1}, characteristic of the OH functional group found in gelatin. The Cantigi leaf extract exhibited an OH absorption peak at 3326.98 cm^{-1}, confirming the presence of phenolic compounds, particularly flavonoids. Additionally, the C=N functional group detected at 1640.66 cm^{-1} in gelatin nanoparticles suggests a cross-linking reaction between the aldehyde group from glutaraldehyde and the amino acid group of gelatin. This indicates a

No.	Bond Type	Wave Number Range (cm^{-1})
1.	-OH	3150-3650
2.	-NH	3000-3500
3.	CH stretching (aliphatic)	2700-3000
4.	C=N	1400-1690
5.	C=O	1650-1900
6.	CH (hydrocarbon)	1300-1475
7.	C=C	650-1000

Table 7: Functional group identification.

Wave number (1/cm)		
Gelatin	**Cantigi leaf extract**	**Gelatin nanoparticles of Cantigi leaf extract**
3280,29	3326,98	3397,92
3074,91	-	-
2960,82	2947,92	2881,99
1625,88	1716,65	1640,66
1625,88	1716,65	1640,66
1334,93	1237,83	1342,07
918,48	916,09	954,29

Table 8: Wave number comparison of gelatin, extract, and nanoparticles.

Schiff base formation, where the amine groups of gelatin react with the aldehyde groups of glutaraldehyde, forming a C=N bond [24].

Antioxidant activity test of nanoparticles using the DPPH method

The antioxidant activity of Cantigi leaf extract gelatin nanoparticles was evaluated based on their IC50 value. The results confirmed that the nanoparticles exhibit very strong antioxidant activity, as their IC50 value was below 50 ppm. A lower IC50 value indicates stronger antioxidant activity. Compared to the IC50 value of Cantigi leaf extract, the IC50 value of its gelatin nanoparticles increased, suggesting that their antioxidant activity was slightly reduced. However, since the IC50 remained below 50 ppm, the nanoparticles still demonstrated strong antioxidant properties. This reduction in activity may be attributed to the presence of additional compounds or excipients in the formulation, which could have influenced the antioxidant potential [8].

Replication	IC50 (ppm)
1	32.10
2	35.74
3	32.90
Mean ± SD	33.58 ± 1.90

Table 9: IC50 of gelatin nanoparticles.

Evaluation of cantigi leaf extract eye cream

Organoleptic examination

The organoleptic evaluation showed that the blank formulation was white, had a cream-like odor, and a smooth texture. No additional fragrance was included, which explains the absence of a strong scent. F1 had a light brown color, characteristic of Cantigi leaf extract, and a soft texture. F2 displayed a light orange color, which resulted from a combination of white base components and the addition of Cantigi leaf extract nanoparticles.

Organoleptic Property	Blank	F1	F2
Color	White	Light brown	Light orange
Smell	Cream-like	Cantigi scent	Cantigi scent
Texture	Smooth	Smooth	Smooth

Table 10: Organoleptic of capsule powder.

Cream type

Methylene blue dye addition caused the inner phase to be colorless and the outer phase to be blue. This color is because methylene blue is soluble in water. The use of stearic acid and cetyl alcohol excipients has an HLB value of 15, and the use of an emulsifier, namely triethanolamine, which is an anionic surfactant that has an HLB of 12, so that the type of cream produced is the oil in water type.

Homogeneity test

The homogeneity test confirmed that all formulations were evenly mixed when applied to a glass slide. This indicates that the

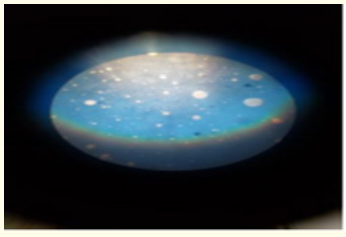

Figure 2: Oil/water cream type of eye cream observed microscopically

excipients and active ingredients were well dispersed within the

No.	Blank	F1	F2
1	Homogeneous	Homogeneous	Homogeneous
2	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous

Table 11: Homogeneity test results.

cream. The blank formulation had a higher pH due to the presence of triethanolamine and stearic acid, which formed an anionic soap with basic properties. F1 and F2 demonstrated stable pH values, remaining within the acceptable range for skin (4.5-6.5). However, F1, which contained Cantigi leaf extract, exhibited a slightly lower pH compared to F2, as Cantigi leaf extract is naturally more acidic than its nanoparticle counterpart. F2 displayed greater spreadability compared to F1, which is due to differences in viscosity.

pH Test

Data on each formula is from the pH test. The blank formulation has a higher pH value than the other two due to the combination of triethanolamine and stearic acid, which produces anionic soap and has basic properties. In F1 and F2, the pH is within the pH requirement range for the skin, namely 4.5-6.5. The pH of F1 containing Cantigi leaf extract has a more acidic pH than F2 containing Cantigi leaf extract-loaded gelatin nanoparticles, and this is due to the pH of Cantigi leaf extract being more acidic than the pH of Cantigi leaf extract gelatin nanoparticles.

Sample	pH
Blank	7.22 ± 0.01
F1	6.06 ± 0.01
F2	6.44 ± 0.02

Table 12: pH measurement.

Sample	Area of Distribution (cm^2)
Blank	31.23 ± 1.03
F1	24.76 ± 3.50
F2	26.56 ± 0.95

Table 13: Spreadability test results.

Spreadability test

Spreadability tests on three formulations are the Blank, F1, and F2. The results of the spreadability test showed that F2 had a greater spreadability than the other formulas. This spreadability is due to the difference in viscosity between formulations. Viscosity is inversely proportional to the ability to spread the cream preparation. Creams have high spreadability, can be used easily without excessive pressure, and have a higher interfacial tension. The results of this test obtained results that met the requirements. The requirements for the spreadability of the cream preparation are 5-7 cm in diameter with a circle area of 19.63 - 38.47 cm^2. F2 exhibited the lowest viscosity among the tested formulations. This difference was attributed to variations in the active substances used-F2 contained nanoparticles, whereas F1used Cantigi leaf extract.

Viscosity and flow properties test

Sample	Viscosity (cps)
Blank	136171
F1	127857
F2	125086

Table 14: Viscosity of eye cream formulations.

The viscosity of the eye cream formulations is affected by pH as the pH of the F1 and F2 are more acidic than that of the Blank. It means that the acidic extract influences viscosity.

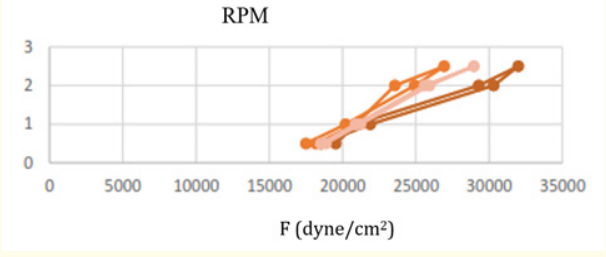

Figure 3: Flow properties of the Blank (right), F1 (left) , and F2 (middle) formulations.

The results indicated that all formulations exhibited thixotropic plastic flow properties, meaning the descending curve appeared to the left of the ascending curve. This behavior is ideal for semi-solid preparations, ensuring high consistency when stored while remaining easy to spread when applied

Antioxidant activity test

The IC50 values of both eye cream formulations were higher (F1 = 48.78 ppm and F2 = 61.87 ppm) compared to the raw Cantigi leaf extract (17.40 ± 0.58) and Cantigi leaf extract gelatin nanoparticles (45.12 ± 0.55). This increase may be attributed to the presence of excipients in the formulations, which could influence antioxidant activity. Despite this change, both eye cream formulations still fell within the strong antioxidant activity category.

Formulation	IC50 (ppm)
F1	48.78 ± 0.95
F2	61.87 ± 0.15

Table 15: Antioxidant activities of F1 and F2.

Irritation Test

Figure 4: Observations of skin irritation on rabbits at time-1h (left) and time-72h.

Treatment	Primary Irritation Index	Irritation Category
Without treatment	0	No irritation
Blank eye cream	0	No irritation
F1	0	No irritation
F2	0	No irritation

Table 20: Primary irritation index.

The irritation test was carried out after the manuscript was evaluated by the Ethics Committee of the Faculty Medicine University of Indonesia-Cipto Mangunkusumo Hospital, Jakarta, Indonesia, of No: KET-1089/UN2.F1/ETIK/PPM.00.02/2023. The irritation test was conducted by assessing erythema and edema responses on the skin of rabbits. Observations were recorded at 1, 24, 48, and 72 hours following sample application and washing. The Primary Irritation Index (PII) results confirmed that all tested formulations, including the blank eye cream, F1 and F2 did not cause irritation. The Cantigi leaf extract and other excipients used were found to be safe for skin application, as none of the formulas triggered adverse skin reactions.

Conclusion

The results showed that the IC_{50} value for Cantigi leaf extract was 17.40 ppm, while for Cantigi leaf extract gelatin nanoparticles, it was 33.58 ppm.

F1 exhibited a light brown color, with viscosity values of 127857 cPs. Its flow properties were classified as thixotropic plastic, and it showed an IC_{50} value of 48.78 ppm, indicating its antioxidant capacity. F2 displayed a light orange color, with viscosity values of 125086 cPs. Similar to F1, its flow properties were also thixotropic plastic. The IC_{50} value was recorded at 61.87 ppm, reflecting its antioxidant potential.

The irritation test demonstrated that both F1 and F2 did not cause skin irritation, based on rabbit skin irritation scores recorded at 1 hour, 24 hours, 48 hours, and 72 hours.

The findings confirmed that Cantigi leaf extract and Cantigi leaf extract gelatin nanoparticles exhibit antioxidant and anti-inflammatory activity. Furthermore, they can be effectively formulated into a stable eye cream that does not cause skin irritation.

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