

Formulation and Evaluation of Gel of Binahong Leaves Nanoparticle Extract (*Anredera cordifolia* (Ten.) Steenis) as an Antioxidant and Sunscreen

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ABSTRACT: Binahong leaves are a herbal plant that is used to cure several diseases in Indonesia. Binahong leaves have flavonoids that can be potentially beneficial for antioxidants and sunscreen. The study aims to formulate an extract and a nanoparticle extract from binahong leaves into a gel and determine antioxidant and sunscreen activity. Binahong leaves were macerated using 70% ethanol and concentrated with a rotary evaporator. Then the extract was made into nanoparticles using the ionic gelation method. The nanoparticles were characterized for particle size, zeta potential, and morphological shape. The results showed that the nanoparticles were 192.7 nm in size, zeta potential of +25.3 mV, and spherical in shape. Antioxidant and sunscreen activity tests were analyzed using a UV-Vis spectrophotometer. Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenger activity method. The binahong leaves extract and nanoparticles had IC₅₀ values of 68.78 ppm (moderate) and 152.18 ppm (weak). The antioxidant gel binahong leaves had IC₅₀ of Formula II (174.85 ppm) and Formula III (221.14 ppm). The sunscreen activity was analyzed by determining the SPF value. Binahong leaves extract and nanoparticles had SPF values of 16.92 and 21.55 (ultra category). The sunscreen gel binahong leaves extract (formula II) and nanoparticles (formula III) had SPF values of 2.61 and 3.3 (minimal category). Gel preparations were evaluated, including organoleptic, homogeneity, spreadability, viscosity, and pH test. Binahong leaves extract and nanoparticles extract had antioxidant activity and SPF value that could be formulated into a gel preparation.

KEYWORDS: Binahong Leaves; Nanoparticle; Gel; Antioxidant; Sunscreen.

1. INTRODUCTION

Indonesia is a tropical country that receives a lot of sunlight, which can affect the skin. This is caused by ultraviolet (UV) rays from the sun, including UV-A and UV-B rays. Excessive exposure to UV rays has harmful effects on human skin, including sunburn and skin aging. When the sun is too strong, the skin must be physically protected by covering the body and chemically protected with sunscreen cosmetics [1].

Repeated exposure to ultraviolet rays causes changes in the structure, composition, and oxidative stress of the skin. A free radical is a compound or molecule that contains one or more unpaired electrons. As a result, electrons that do not have a partner become very reactive in finding a partner by attacking and binding electrons around them so that they can cause various diseases. Antioxidants are compounds that can inhibit, prevent, and neutralize the oxidative damage caused by free radicals following exposure to UV light [2].

Sunscreens protect the skin from the adverse effects of UV rays by absorbing, reflecting, and/or scattering the UV rays. To achieve maximum protection, sunscreen should contain a Sun Protection Factor (SPF) value that is sufficient to protect the skin from UV exposure, which is SPF 15-50. SPF is the value used to measure the effectiveness of sunscreen protection against UV B rays.

Various sunscreen innovations have been developed by adding natural ingredients that have the potential to increase the SPF value as active substances. The ability of natural ingredients to protect the skin from UV rays through contained compounds that have the potential as photoprotective sunscreens, namely phenol compounds, is supported by the presence of compounds that are antioxidants, such as flavonoids [3].

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Flavonoids have aromatic rings that can absorb UV A and UV B rays at a wavelength of 200-400 nm to protect the skin from UV radiation [4]. One of the plants that contains flavonoids and has not been widely studied to be formulated into antioxidant and sunscreen cosmetics is binahong leaves (*Anredera cordifolia* (Ten.) Steenis).

Nanoparticle innovation can be used to maximize the effectiveness of the preparation. Nanoparticles can be dispersed at the nanoscale (10-1000 nm). The implementation of nanoparticles in the pharmaceutical field involves the delivery of drugs in a nanometer-sized carrier system.

The preparation of binahong leaf nanoparticles was based on the ionic gelation method [5]. The ionic gelation method involves cross-linking between polyelectrolytes in the presence of multivalent ion pairs [6]. Chitosan and sodium alginate are two biopolymers that can entrap therapeutic agents (flavonoids), maintain their structure and activity, and protect them from metabolic (enzymatic) degradation [7].

The aim of this study was to develop nanoparticulate systems based on ionic gelation between chitosan and sodium alginate for the loading of binahong leaf extract and to compare the antioxidant and sunscreen activities of binahong leaf extract and binahong leaf extract nanoparticles. The obtained nanoparticles were characterized in terms of their size, zeta potential, and morphology..

2. MATERIALS AND METHODS

2.1. Materials

Chitosan, sodium alginate, propylene glycol, Tween 80, Aquadest, glycerin, ethanol 70%, ethanol 96%, methanol, vitamin C, DPPH (2,2-diphenyl-1-picrylhydrazyl).

2.2. Methods

2.2.1. Preparation of the Extraction of Binahong Leaves

The preparation of binahong leaf extract nanoparticles was carried out by a maceration technique using 70% ethanol solvent with a ratio of material to solvent of 1 kg of simplisia powder with 6 liters of 70% ethanol solvent for 2 × 24 h while stirring for the first 2-3 hours. After the maceration process was completed, it was filtered using a filter paper to remove the filtrate and separate the pulp. The macerate was concentrated with a rotavapor at 40 °C for 6 h to obtain a thick extract, which was weighed.

2.2.2 Preparation of Binahong Leaves Extract Nanoparticle Formulas

Synthesis of Nanoparticles

Binahong leaf extract (800 mg) was dissolved in Tween 80, 96% ethanol, propylene glycol, and aqueous solution. Afterward, 18 mL of chitosan 0.5% was added and gradually homogenized with a magnetic stirrer. Sodium alginate (0.1%, 30 mL) was added dropwise and homogenized using a magnetic stirrer for 60 min at 300 rpm. The obtained nanoparticles were characterized in terms of their size, zeta potential, and morphology.

Table 1. The formula of extract binahong leaves nanoparticles

Materials	Concentra-tion (v/v)	Function
Binahong Leaves Extract (%)	0.8	Active material
Sodium Alginate 0.1%	30	Crosslink agent
Chitosan 0.5%	18	Crosslink agent
Propylene glycol	5	Cosolvent
Ethanol 96%	15	Cosolvent
Tween 80	6	Cosolvent
Aquadest	Ad 100 mL	Cosolvent

Formulation of gel binahong leaves

The gel of the binahong leaves was formulated into two different formulas: antioxidant gel and sunscreen gel.

Table 2. Formula antioxidant gel binahong leaves extract and nanoparticles extract

Material	Formulation gel binahong leaves (%)			
	F I	F II	F III	Function
Binahong leaves extract	-	100 x IC ₅₀	-	Active material
Binahong leaves nanoparticle extract	-	-	100 x IC ₅₀	Active material
Na-CMC	2	2	2	Gelling agent
Carbopol 940	0.5	0.5	0.5	Gelling agent
Triethanolamine	0.25	0.25	0.25	Alkalizing agent
Propylene glycol	15	15	15	Solvent agent
Phenoxyethanol	0.5	0.5	0.5	Preservative agent
Aquadest Ad	100	100	100	Solvent agent

Table 3. Formula sunscreen gel binahong leaves extract and nanoparticles extract

Material	Formulation gel binahong leaves (%)			
	F I	F II	F III	Function
Binahong leaves extract	-	0.1	-	Active material
Binahong leaves nanoparticles extract	-	-	0.1	Active material
Carbopol 940	0,5	0,5	0,5	Gelling agent
Triethanolamine	0,25	0,25	0,25	Alkalizing agent
Glycerin	10	10	10	Solvent agent
Phenoxyethanol	0,5	0,5	0,5	Preservative agent
Aquadest Ad	100	100	100	Solvent agent

Description:

Formula I: blanko gel

Formula II: gel of binahong leaves extract

Formula III: gel of binahong leaves nanoparticles extract

The composition of the antioxidant gel binahong leaves is shown in Table 2. Gel was formulated with a combination of gelling agents such as Na-CMC and Carbopol. The gel formulation started by heating 10 mL of water to dissolve Carbopol 940 and 40 mL of water to dissolve Na-CMC. Triethanolamine and Carbopol 940 were then added to a beaker above a stirrer and stirred quickly until they were dissolved and homogeneous. Na-CMC and carbopol were mixed above the stirrer using a beaker containing a mixture of phenoxyethanol, propylene glycol, and binahong leaf extract while continuously stirring until homogeneous. until homogeneous.

Sunscreen gel was prepared by slowly adding Carbopol 940 to water and mixing until a lump-free dispersion was obtained. Dissolved binahong leaf extract into glycerin. The extract solution and nanoparticle solution of the binahong leaf extract were added to phenoxyethanol and stirred until homogeneous (mixture 1). Then, Carbopol 940 was stirred using a homogenizer and neutralized with triethanolamine (mixture 2). Mixture 1 was added gradually into mixture 2 using a homogenizer, and then glycerin and aqueous solutions

were added gradually. Subsequently, the gel that had been formed was placed into the tube. Then, the evaluation of the gel and its SPF value was tested.

2.2.3. Evaluation of gel binahong leaves

Organoleptic test

The prepared gel formulation was inspected visually for color, odor, and texture.

Homogeneity test

Homogeneity tests were carried out on gels that had been made before and after storage. The gel was applied to a piece of glass or another suitable transparent material, and the homogeneity of the preparation was observed.

Viscosity test

The viscosity test was performed on the gel using a Brookfield Viscometer RV. This was performed by dipping the spindle into the gel preparation and then observing and calculating the viscosity.

Spreadability test

The spreadability test is important as it shows the behavior of the gel exiting the tube. The diameters of the spread circles range from 5 to 6.5 cm.

pH test

The pH test was performed using a HANNA Instrument HI 2211 pH meter by taking 0.5 g of gel that had been diluted into 50 mL of distilled water, measuring with a pH meter, letting it stand for a few moments, and the results are seen in the description of the pH meter. Preparations that meet the skin criteria are in the interval 5-7.

2.2.4. Determination of antioxidants using the DPPH method

The antioxidant activity of the extract, extract binahong leaf nanoparticles, and gel binahong leaves was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenger activity method. The absorbance reading at 516 nm (in triplicate) was performed using a UV-Visible spectrophotometer (Shimadzu 1900).

$$\text{Inhibition (\%)} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\%$$

The IC₅₀ value was obtained from the intersection of the lines between the inhibition power and the concentration, and was then substituted into the equation $y = a + bx$, where y for 50 and x value for IC₅₀.

The antioxidant activity was expressed as IC₅₀, which indicates the antioxidant concentration required to quench 50% of the initial DPPH radicals under the given experimental conditions.

Gel preparation contained binahong leaves with 3 formulas: each formula contained blanko, binahong leaves extract and binahong leaves nanoparticles. The concentration formula II and formula III is $100 \times \text{IC}_{50}$ from the extract and nanoparticle extract.

2.2.5. Determination of Sun Protection Factor (SPF) value

The SPF value test of the extract, binahong leaf extract nanoparticles, and gel binahong leaves was performed using the UV-Vis spectrophotometric method by preparing a 1000 ppm solution of binahong leaf extract and nanoparticle extract in 10 mL of 96% ethanol:aquadest (2:1) and measuring the absorbance every 5 nm in the wavelength range of 290-320 nm. The SPF value was determined using the equation developed by Mansur. The equation used was as follows:

$$\text{SPF} = \text{CF} \times \sum_{320}^{290} \text{EE} \times \text{I} \times \text{Abs}$$

Description:

CF = Correction factor

EE = Spectrum of erythema effects

I = Solar intensity spectrum

Abs = Sample absorbance

The correction factor values and erythema effect spectrum are shown in the following table:

Table 4. EE x I value to determine SPF value

Wavelength	EE x I value
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

SPF values range from 0 to 100, and the ability of sunscreens that are considered good is above 15. According to the Food and Drug Administration, sunscreen) is as follows [8]:

Table 5. SPF value classification

Type of protection	SPF value
Minimal	1-4
Moderate	4-6
Extra	6-8
Maximal	8-15
Ultra	>15

3. RESULTS AND DISCUSSION

3.1. Characterization of bina-hong leaves extract nanoparticles

3.1.1. Size and distribution particles

The particle size range of the nanoparticles was 10-1000 nm. The results of particles on binahong leaves nanoparticles extracted using the Malvern Zeta Sizer showed that the formed nanoparticles had an average size of 192.7 nm. The measurement results showed that the nanoparticles were of good quality.

3.1.2. Zeta potential

The zeta potential was +25 mV. The charge possessed by the nanoparticles is far from the value of 0 and is almost close to +/- 30 mV, so nanoparticles are obtained that are stable and do not easily aggregate.

3.1.3. Particle morphology

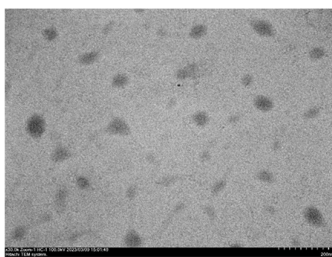


Figure 1. Nanoparticle morphology

Based on TEM observations of nanoparticles at 10,000 × magnification, it is known that nanoparticles are spherical. Surface morphology affects the ability of nanoparticles to penetrate the target cell membrane. The spherical surface of nanoparticles can enter cells more easily [9].

3.2. Evaluation of gel binahong leaves

3.2.1. Antioxidant gel binahong leaves

Organoleptic test

Table 6. Results of the antioxidant gel organoleptic test

Test	F I	F II	F III
Color	Clear	Dark green	Light green
Odor	Odorless	Aromatic binahong leaves	Aromatic binahong leaves
Texture	Semi solid	Semi solid	Semi solid

Sensory evaluation was conducted on the gel samples from each formulation, which varied from odorless to aromatic binahong leaves. The textures of the gel samples from all formulations were semi-solid. The color of the gel varied significantly depending on the content of the active material used, ranging from clear to light green. The color, odor, and texture of the gel sample were derived from the binahong leaves.

Homogeneity test

Table 7. Results of the antioxidant gel homogeneity test

F I	F II	F III
Homogeneous	Homogeneous	Homogeneous

Each gel formulation showed no visible presence of coarse particles on the glass slide during observation and had a uniform color. These gels were considered homogenous.

Spreadability test

Table 8. Results of the antioxidant gel spreadability test

F I	F II	F III
5.59 cm	5.87 cm	6.44 cm

Based on the spreadability test, formula I had an average spread of 5,59 cm, formula II had an average spread of 5,87 cm, and formula III had an average spread of 6,44 cm. This shows that viscosity affects the spreadability of the gel preparation, where the lower the viscosity value, the higher the spreadability.

Viscosity test

Table 9. Results of the antioxidant gel viscosity test

F I	F II	F III
146600 cPs	128940 cPs	85320 cPs

Formulas I, II, and III had significant differences in viscosity values of 146600 cps, 128940 cps, and 85320 cps, respectively. This was due to the addition of the extract and the binahong leaf nanoparticle extract. Based on the organoleptic test, the extract has a texture similar to a viscous mass, and the nanoparticles have a liquid form. This resulted in gel formula III having a lower viscosity value.

The concentrations of Na-CMC and carbopol are important components for the formation of a gel base as a gelling agent. The higher the concentration of Na-CMC, the thicker the consistency of the resulting gel preparation, and the concentration of Carbopol can increase the viscosity of the gel owing to neutralization of the gel preparation with the addition of triethanolamine.

pH test

Table 10. Results of the antioxidant gel pH test

F I	F II	F III
6.47	6.27	5.32

The gel binahong leaves from each formula varied in pH. The pH varied significantly, depending on the active material content. Formula I was the highest pH value from all formulas. This was due to the blank not containing binahong leaves, so that it was a pH close to neutral. The addition of binahong leaf extract to formula II caused a decrease in the pH of the preparation owing to the presence of binahong leaf extract, which contained tannins and flavonoids, which are phenolic compounds.

Formula III which contained binahong leaves nanoparticles extract had a lower pH than formula II because binahong leaves nanoparticles extract has an acidic pH of 4.58. When the pH of the gel was below 4.5. It becomes acidic and irritates the skin. When the pH of the gel is above 7, it becomes alkaline and can cause skin dryness and scaling.

3.2.2. Sunscreen gel binahong leaves

Organoleptic test

Table 11. Results of the sunscreen gel organoleptic test

Test	F I	F II	F III
Color	Clear	dark green	light green
Odor	odorless	aromatic binahong leaves	aromatic binahong leaves
Texture	semi solid	semi solid	semi solid

All formulas had a semi-solid form. Formula I has a clear color and no odor, whereas formulas II and III have a dark green color with an aromatic odor of binahong leaves. Because in formula I there was no additional extract or nanoparticle extract, the color was clear and odorless, while the green color with an aromatic odor of binahong leaves in formulas II and III was the color of the extract and nanoparticle extract.

Homogeneity test

Table 12. Results of the sunscreen gel homogeneity test

F I	F II	F III
Homogeneous	Homogeneous	Homogeneous

All formulas that the gel has been made are homogeneous, so that it can be seen that the active substances and additives can mix evenly. It is expected that with a homogeneous gel, the active substance can be spread evenly in the gel, and the right concentration is obtained when used.

Spreadability test

Table 13. Results of the sunscreen gel spreadability test

F I	F II	F III
5.54 cm	5.95 cm	6.18 cm

Formula III has a greater spreading ability than formula I and formula II. This could be caused by the differences in pH and gel viscosity. When the pH of the gel decreases, the viscosity of the gel also decreases and the spreadability of the gel increases [10]. The wider the spreadability of the gel, the wider the gel protects the skin.

Viscosity test

Table 14. Results of the sunscreen gel viscosity test

F I	F II	F III
272200 cPs	228100 cPs	166400 cPs

Formula II and formula III obtained viscosity results that decreased. This could be caused by the influence of the pH of the gel, which tends to be acidic. The pH affects the physical properties of the gel. The more the pH of the gel decreases, the more the viscosity of the gel decreases. Acidic pH affects the gel base, which has an impact on decreasing the viscosity of the gel.

pH test

Table 15. Results of the sunscreen gel pH test

F I	F II	F III
6.03	5.82	5.74

Formula I had a higher pH value than formula II and III because formula I did not contain binahong leaves extract and nanoparticles. The pH change in formula II could occur due to the addition of binahong leaf extract and acidic solvents. The pH of the gel decreased because binahong leaf extract contained flavonoids, which are phenolic compounds that can cause a decrease in pH. However, in formula III, there was a change in pH due to the binahong leaf nanoparticle extract, which has an acidic pH of 4.58, so the pH in formula III was lower than that in formula II.

3.3. Antioxidant using DPPH method

Table 16. IC₅₀ value sample

Sample	IC ₅₀ (ppm)	Requirement
Vitamin C	3.9	
Binahong extract leaves	68.78	<50 = Very strong 50-100 = Strong 101-150 = Moderate 151-200 = Weak
Binahong leaves nanoparticle extract	152.18	

In this test, vitamin C was used as a positive control because vitamin C is a very strong antioxidant. Based on the test results, the IC₅₀ value of vitamin C was 3.9 ppm. The antioxidant activity can be seen from the IC₅₀ value; the smaller the value, the stronger the antioxidant activity.

Binahong leaves extract demonstrated an IC₅₀ value of 68.79 ppm which means the antioxidant activity was strong. This can be caused by the content of secondary metabolites contained in binahong leaves, which have flavonoids in the flavonol group. Flavonoids can inhibit oxidation by donating H atoms, which bind free radicals to produce compounds that are more stable.

The IC₅₀ value of the nanoparticles was 152.18 ppm which indicated that the antioxidant activity was moderate. This is due to the use of nanoparticle ingredients, which cause the extract to be absorbed on the formed nanoparticles so that the release of flavonoids becomes slow and the solvent can inhibit absorption.

Table 17. IC₅₀ value gel preparation

No	Sample	IC ₅₀ (ppm)	Requirement
1.	Formula II	174.85	<50 = Very strong 50-100 = Strong 101-150 = Moderate 151-200 = Weak >200 = Very weak
2.	Formula III	221.14	

Based on the results of activity tests on gel binahong leaves, formula II and formula III had antioxidant activity of 174.85 ppm and 221.14 ppm. Formula II is stronger than formula III in inhibiting free radicals.

Based on the IC₅₀ value, the binahong leaf extract has stronger activity than the binahong leaf nanoparticle extract, which causes the formula II gel preparation to be stronger. Based on the concentration in each formula calculated based on IC₅₀, formula II contained 0.69% binahong leaf extract, while formula III contained 0.15% binahong leaf nanoparticle extract.

Sun Protection Factor (SPF) value

Table 18. SPF value of extract, binahong leaves nanoparticles extract and gel binahong leaves

Formula	SPF Value
Binahong leaves extract	16.92
Binahong leaves extract nanoparticles	21.55
Gel of formula II	2.61
Gel of formula III	3.31

Based on the calculation of the SPF value test of binahong leaf extract, the average SPF value is 16.92, which is in the ultra category. Binahong leaves extract contains a group of phenols, one of which is flavonoids which can provide activity as a sunscreen. Conjugated aromatic rings in flavonoid compounds are able to absorb UV B radiation, which can reduce the adverse effects of UV radiation on the skin, so that when binahong leaf extract was measured using a UV-Vis spectrophotometer, they can provide absorption at UV wavelengths [11].

Meanwhile, the calculation of the SPF value test of binahong leaf extract nanoparticles resulted in an average SPF value of 21.55, which is in the ultra category. Determination of the SPF value of binahong leaf nanoparticle extracts with the same concentration as binahong leaf extract showed a higher SPF value compared to the binahong leaf extract solution. The binahong leaves nanoparticles extract as a sunscreen has small particles so that with the same concentration but in different shapes, the nanoparticles demonstrated their active compounds of sunscreens move freely, which can increase the absorption at each wavelength tested so as to increase the SPF value of the active substances used [11].

From the results of the SPF value examination, it was found that the SPF value of the binahong leaves nanoparticles extract sunscreen gel (Formula III) was higher than the binahong leaves extract sunscreen gel (Formula II). The SPF value of the binahong leaf nanoparticle extract gel increased because the nanoparticle extract had smaller particles than the extract, and in the nanoparticle solution, there were many variations of solvents that increased the absorption value of the test. The SPF values of the gel of the extract and binahong leaf nanoparticles extract were in the minimal protection category. The SPF value of the gel decreased compared to that of the binahong leaf extract solution and the binahong leaf extract nanoparticle solution. This could be due to the addition of solvents to dissolve the extract and dilution at the time of testing, which could have affected the SPF value.

4. CONCLUSIONS

The nanoparticles generated by the interaction between chitosan and sodium alginate had a size of 192.7 nm and zeta potential of +25 mV. Binahong leaves extract and nanoparticles had IC_{50} values of 68.78 ppm (strong) and 152.18 ppm (moderate). The antioxidant gel binahong leaves had IC_{50} values 174.85 ppm (formula II) and 221.14 ppm (formula III), which classified weak.

Binahong leaves extract and nanoparticles had SPF values of 16.92 and 21.55, which were in the ultra category. The gel of binahong leaf extract (formula II) and nanoparticles (formula III) had SPF values of 2.61 and 3.31, respectively, which belonged to the minimal category. The binahong leaf extract and nanoparticle extract can be formulated into gel preparation and qualified.

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