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Formulation and Evaluation of Gelatin Nanoparticle Moisturizing Gel from Mesocarp Extract of Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] as an Antioxidant

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Abstract

Human skin might get attacked by free radicals therefore, antioxidants are needed. Mesocarp of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] has natural antioxidant compounds such as citrulline. Antioxidant compounds were formulated into gel preparation with nanoparticle technology that aimed to facilitate the absorption of an active substance. The purpose of this study was to formulate an antioxidant moisturizing gel that contained gelatin nanoparticles of mesocarp extract of watermelon (GNMW). The stages of research included the extraction, production, and characterization of nanoparticles, formulation of gel preparations with various concentrations of 0.0114% (F1), 0.0228% (F2), and 0.0342% (F3), evaluation of gel preparations, observation of antioxidant activity using DPPH method and skin moisture test. The nanoparticles produced had an irregular shape, size of 200.3 nm, polydispersity index of 0.288, and zeta potential value of +16.11 mV. The nanoparticle gel had a slightly viscous texture, clear color, and homogeneous, with a pH value of 6.61 – 7.22; viscosity of 14740 – 17180 cP; spread-ability of 5.3-6.5 cm; and did not irritate the skin. The results of the antioxidant activity of the extract and nanoparticles were 110.90 µg/mL and 114.16 µg/mL, respectively. Nanoparticle gel of F1, F2, and F3 had IC_{50} values of 149.52 µg/mL; 138.44 µg/mL; and 127.10 µg/mL, respectively. The gels could increase skin moisture content in the range of 38.81% - 63.19%. Mesocarp extract of watermelon which was made into nanoparticles with gelatin carrier could be formulated into a moisturizing gel, met good gel parameter standards, and had moderate antioxidant activity.

Keywords: Nanoparticle moisturizing gel; GNMW; Nanoparticle antioxidant gel.

Introduction

The skin is the outermost part that covers all parts of the body. Antioxidants are needed to protect the body from free radical attacks such as UV rays. One natural product that has the potential as a natural antioxidant and is believed to neutralize free radical in the human body is watermelon (*Citrullus lanatus*). This fruit is in high demand because of its sweet and fresh taste and can be functioned as a skin moisturizer. Due to the high demand for watermelon consumption, the large amount of watermelon peel waste has also increased. Waste produced from watermelon white peel (mesocarp) is about 30% per fruit (1). The mesocarp of watermelon (MW) has a natural antioxidant compound in the form of citrulline, which is higher than its endocarp (flesh of

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watermelon) by 60% or about 15.6 mg/g dry weight.

MW was reported to contain flavonoid compounds that can act as an antioxidant also with an IC_{50} value of 31.42 µg/mL (2). One of the topical dosage forms that have been developed is gel. A gel is a semisolid-shaped preparation made from small inorganic particles or large organic molecules and penetrated by a liquid. One of the important factors in gel formulation is the selection of a gelling agent. Fujiastuti & Sugihartini have formulated a gel preparation contained *Centella asiatica* L extract in various type of gelling agent such as HPMC, Carbopol and CMC sodium. The result showed that Carbopol was the best type of gelling agent because it gave the strongest adhesive power and minimal irritation effects (3). Carbopol expands when dispersed in air in the presence of alkaline substances such as triethanolamine or di-isopropanol amine to form semisolid preparations. Carbopol behaves like a non-Newtonian fluid, but as long as the shear stress is below certain values, the gel will not flow and reversible, elastic deformation will occur (4).

Nowadays, many topical dosage industries compete for the maximum ability of the active substance. Nanoparticle technology has become a new trend in the development of drug delivery systems because it has the advantage that is easier to pass through intracellular spaces.

Mahmoudi et al. showed that the modification of *Physalis alkekengi* L. extract into nanoparticle could increase antioxidant activity (5). Biopolymers that have been widely used as carriers in nanoparticle technology are gelatin because they have good biocompatibility and biodegradability properties. Gelatin is a protein obtained from the hydrolysis of collagen. Gelatin has several advantages, such as low cost, easy bioavailability, opportunities to be combined with crosslinkers, easily decomposed, and has the ability to interact with cells without causing toxicity (6). Nejat et al. showed that gelatin could be used to prepare cardamom extract-loaded gelatin nanoparticles as an effective targeted drug delivery system to treat glioblastoma (7).

Formulation of gelatin nanoparticles containing mesocarp extract of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is of great necessity. This study aimed to utilize watermelon peel waste through the formulation of a gel preparation that has an antioxidant activity which is made into a nanoparticle preparation with a gelatin carrier. To the best of our knowledge, no studies have previously been conducted on the formulation of gelatin nanoparticles containing mesocarp extract of watermelon as an antioxidant for gel preparation. Measurement of antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-picrylhidrazyl) method. The DPPH method was chosen because it is simple, easy, fast, sensitive, and requires a small number of samples.

Materials and Methods

1. Chemicals and plant materials

DPPH (2,2-diphenyl-1-picrilhydrazyl), ascorbic acid, DMSO (Dimethyl Sulfoxide) and acetone solvent were purchased from Sigma Aldrich (USA). Other chemicals were obtained from the chemical warehouse of the Pharmacy Department, Sekolah Tinggi Ilmu Kesehatan Bani Saleh with pharmaceutical grade. Mesocarp of watermelon sample was obtained from fresh watermelons at watermelon plantations in Saptomulyo Village, Kota Gajah, Lampung Province, Indonesia.

2. Simplicia preparations

Fresh watermelon fruits were cut and separated between the mesocarp and endocarp. MW then washed, cut into cubes with the size of 1 cm x 1 cm x 1 cm, and dried under indirect sunlight for 7 days. Dried MW was then crushed until a powder of certain fineness was obtained (2). Powdered MW was macroscopically observed, including color, odor, and shape, and then observed the fineness degree using a sieving pan.

3. Extraction of the simplicia

Specific weights (1.20 kg) of powdered MW were macerated using 70% ethanol in ratio of 1:10 for 2x24 hours with occasionally stirred, then stored in a dark or sun-tight room and filtered. The residue was then re-macerated and the filtrates in each maceration process were gathered and evaporated using a rotary evaporator until a concentrated extract of MW was obtained (8).

4. Characterization of extract

Water content, pH, solubility, and phytochemical screening were carried out toward the extract of MW.

5. Phytochemical screening

Phytochemical screening including alkaloids, flavonoids, saponins, steroids/triterpenoids and tannins was carried out toward the extract of MW.

A specific amount (0.6 g) of MW extract was added 3 mL of 2 N HCl solution, then divided into 3 test tubes. Mayer's, Dragendorff's, and Wagner's reagent were added to each tube sequentially, then the changes that occurred were observed. Alkaloids are positive if there is a precipitate or color change in at least two of the three experiments above. The positive reaction of alkaloids is the formation of a yellow or white precipitate with Mayer's reagent, an orange precipitate with Dragendorff's reagent, and the formation of a reddish-brown color with

Wagner's reagent (9).

A specific amount (0.2 g) of MW extract was added with 1 mL of concentrated HCl and 0.2 g of Mg powder. The positive reaction of flavonoids is indicated by the formation of red, orange, or yellow color (9).

A specific amount (0.5 g) of MW extract was added 10 mL of hot water, cooled, then shaken vigorously for 10 seconds. The positive reaction of saponins is indicated by the formation of 1 – 10 cm foam which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl (9).

A specific amount (0.2 g) of MW extract was added with Liebermann-Burchard reagent. The positive reaction of steroids is indicated by the formation of a greenish blue color whereas the positive reaction of triterpenoids is indicated by the formation of purple color (9).

A specific amount (0.1 g) of MW extract was added 10 mL of hot water, boiled, and filtered. To the filtrate, 2 drops of gelatin 1% was added. The positive reaction of tannins is indicated by the formation white precipitate (9).

6. Gelatin nanoparticle of MW preparation

Gelatin nanoparticle was made by the desolvation method as described by Coester et al. with modification (10)**.** A specific amount (100 mg) of MW extract was dissolved in a mixed solvent (15 mL of ethanol and 10 mL of DMSO). A specific amount (100 mg) of gelatin was dissolved in 25 mL of water with constant heating at a temperature of 40 ^{o}C using a magnetic stirrer at speed of 1000 rpm until a clear solution was obtained. The gelatin solution was then adjusted its pH until 3 with the addition of 0.1 M HCl or 0.1 M NaOH solution. MW extract solution was then added to the gelatin solution followed by the addition of Pluronic F-68 (300 mg) while stirring continuously using magnetic stirrer at speed of 1000 rpm. 30 mL acetone were then added drop by drop. After 10 minutes, 0.2 mL of glutaraldehyde was added then stirred for 3 hours at a temperature of 40 ^{o}C to bind the nanoparticles. Nanoparticles were then purified by being placed into a dialysis tube soaked with water for 24 hours. Furthermore, nanoparticles were dried using freeze dryer at a constant temperature of -45 ^{o}C (11). An organoleptic test of gelatin nanoparticle of MW (GNMW) was carried out.

7. GNMW characterization

GNMW was characterized by its particle size and polydispersity index using a Particle Size Analyzer at 20^{o} C (12), zeta potential, morphology using Scanning Electron Microscope, solubility, and functional group using Fourier Transform Infrared Spectrophotometer.

8. Gel preparation

Water was heated at a temperature of 70 ^{o}C. A specific weight (0.5 mL) of Carbopol 940 was developed with 20 times of hot water for 30 minutes, then stirred quickly in the mortar until a gel mass was formed. Specific weights (2 g) of TEA (triethanolamine) were added and then ground until homogenous. A specific weight (0.5 mL) of phenoxyethanol was dissolved with 15 mL of propylene glycol and 0.1 g of sodium metabisulfite with 5 mL of water, then placed in the mortar and stirred until homogenous. GNMW was dissolved with the remaining water, placed in the mortar and stirrer until homogenous then added with water until 100 g (13). Formulation of GNMW gel is shown in Table 1.

9. Evaluation of GNMW gel

Evaluation of GNMW gel included organoleptic test, pH, homogeneity, viscosity, spread-ability, irritation test, humidity test, and antioxidant activity test using the DPPH method.

10. Irritation test

Irritation test was performed on 10 volunteers (male and female), who were selected by age criteria of 20-35 years old and never had skin problems before (14). GNMW gel of F0, F1, F2, and F3 were applied on the upper forearm, covered with gauze, and plastered. After being left for 1 hour, the plaster was opened and seen for the symptoms caused such as itching, redness, or

Table 1. Gel formulation of GNMW

Substances	Formulation				Function
	F0	F1	F2	F3	
GNMW (%)	-	0.0114	0.0228	0.0342	Active ingredients
Carbopol 940 (%)	0,5	0,5	0,5	0,5	Gelling agent
TEA (%)	1	1	1	1	Buffer
Propylene glycol (%)	15	15	15	15	Humectant
Phenoxyethanol (%)	0,5	0,5	0,5	0,5	Preservative
Sodium metabisulfite (%)	0,1	0,1	0,1	0,1	Antioxidant
Water (%)	ad 100	ad 100	ad 100	ad 100	Solvent

swelling (15).

11. Skin moisture test

A skin moisture test was performed on 10 volunteers. Volunteers were conditioned at room temperature for 30 minutes (16). Five areas of 6 cm^2 were marked (17). Before applying the GNMW gel, a moisture test was conducted on dull forearm skin. GNMW gel of F0, F1, F2, and F3 were applied on the forearm. The negative control was the dull forearm skin. Skin moisture was measured hourly for 3 hours using Skin Moisture Analyzer (18).

12. Antioxidant activity assay

Antioxidant activity assay was carried out toward MW, GNMW, and GNMW gel preparations using vitamin C as the positive control. A specific amount of samples (1 mL) were added by 1 mL of DPPH solution and 3 mL of ethanol, homogenized by a vortex mixer for 1 minute then incubated at 37 ^oC for 30 minutes. Absorbance was measured at a maximum wavelength of 517 nm with a UV-Vis Spectrophotometer. The experiment was performed 3 times for replication. The IC_{50} value was obtained from linear regression equation $y= ax + b$ where x was the concentration value of the sample solution and y was the percent inhibition against DPPH (19).

13. Statistical analysis

To estimate the statistical significance of the measured values, ANOVA (Analysis of Variance) was performed using SPSS v. 22 including the data of normality, homogeneity, spread-ability, pH, viscosity and antioxidant activity. The data obtained from antioxidant activity tests were calculated for IC_{50} value using linear regression method.

ANOVA was performed to estimate the statistical significance of the measured values, including IC_{50} value. Shapiro-Wilk test was performed to determine the normality of the data, while Levene statistical test was performed to determine the homogeneity of the data.

Results and Discussion

1. Simplicia preparations

A total amount of 27.63 kg of MW was cut into cubes to support the drying process. The drying process aimed to extend the shelf life of Simplicia. Dried MW was crushed to produce smaller particle sizes so the extraction process would be more effective. The finer the simplicia powder produced, the larger the surface area of the powder, so the higher the contact between the powder and the solvent. The dried Simplicia obtained was stored at a temperature of 15-30 ^oC. Dried MW was a powder, brownish-yellow color, and had a characteristic odor.

2. Extraction of the simplicia

Maceration was carried out for the extraction process. The principle was "*like dissolved like"*, which meant that a compound would be dissolved in a solvent with the same properties. The maceration method was chosen because of the simple and practical extraction process. Stirring during the maceration process was carried out to homogenous the concentration of the solution and could optimize the secondary metabolite retrieval. In the maceration process, Simplicia would undergo a breakdown of the cell wall due to the pressure difference between inside and outside the cell, so that the secondary metabolites present in the cytoplasm would be dissolved in organic solvent (20). 70% ethanol was used as a solvent in the maceration process because it could attract both polar and nonpolar compounds, and is nontoxic compared to other solvents. Remaceration was carried out to fully retrieve the secondary metabolite contained in the sample. The filtrate obtained was a solid green color. The macerates were then evaporated to produce a concentrated extract. The final result of this extraction process was a reddish-brown thick extract of MW with a yield of 25.025%. MW extract was viscous, reddish-brown in color, and had a characteristic odor.

3. Characterization of extract

The water content of the MW extract was 19.51% which met the standard value of good water content for viscous extracts (5-30%). High water content could facilitate microbial growth.

The pH of MW extract was obtained at 5.13 which tent to be acidic. The optimum pH value for the production of gel preparation is 4-7. Too high a pH value would cause the gel to be less stable.

A solubility test was carried out to determine the degree of solubility of extracts in various solvents as well as to determine the polarity of extracts that would have an effect when selecting solvents for the manufacture of nanoparticle and gel preparations.

Based on the results of the solubility test, MW extract was very soluble in water and propylene glycol, soluble in ethanol and DMSO, and very slightly soluble in acetone.

Phytochemical screening was necessary to identify the class of secondary metabolite compounds of GNMW extract. The phytochemical screening results stated that MW extract contained alkaloids, flavonoids, and saponins compounds. The result is shown in Table 2.

Table 2. Secondary metabolites screening result of MW extract

Secondary metabolite compound	Reagent	Result
Alkaloid	Mayer	-
	Dragendorff	+
	Wagner	+
Flavonoid	Wilstatter	+
Tannin	$FeCl_3$ 1%	-
Saponin	Water	+
Steroid	Liebermann-Burchard	-
Terpenoid	Liebermann-Burchard	-

(+) : Contain secondary metabolite compound
(-) : Did not contain secondary metabolite compound

4. GNMW preparation

The production of GNMW was carried out by the desolvation method which is a bottom-up type nanoparticle formation method. The synthesis of nanoparticles was conducted from small molecules size and then forming larger particles using chemical methods. The desolvation method was chosen because it was more economical, non-toxic, and easy to apply by using two types of solvents to avoid damaging factors including increased flow rate, heating, and stirring which could interfere with the tertiary structure of the protein from gelatin. The extract was dissolved with a mixed solvent in the form of ethanol and DMSO, aimed to dissolve the secondary metabolite compounds contained in the extract. A mixed solvent was used to completely dissolve the extract with gelatin solution which could easily attach to the nanoparticles formed (21).

Gelatin is a protein obtained through the hydrolysis of collagen from the skin, white connective tissue, and cartilage. The gelatin used was type B gelatin, because it has better potential in terms of delivery (4). Type B gelatin is obtained from bovine bones through alkaline hydrolysis which has an isoelectric point (IEP) value of 4-5 (22). Gelatin is denatured at temperatures above 37 ^oC but its molecular weight also does not change at that temperature so this study was performed at a temperature of 40 ^oC to dissolve gelatin with water (23).

The extract solution was then mixed into the gelatin solution. Production of GNMW was carried out using a magnetic stirrer at a temperature of 40 ^oC and a speed of 1000 rpm. The increasing speed of stirring, the smaller the particle size and the low value of the polydispersity index formed, due to the increased surface stabilization to prevent aggregation (11). Pluronic F-68 was then added to the mixed solution which functions as a surfactant that could increase the dissolution rate of the active substance by lowering the aggregation of active substance particles, resulting in good stability. The mixed solution of MW extract, gelatin, and pluronic F-68 was then set at its pH until 3. The pH adjustment aimed to keep the polymer away from its isoelectric point so that gelatin did not easily form aggregates allowing for an increase in zeta potential. The smaller the pH value, the smaller the size of the nanoparticle because the maximum solubility of gelatin was at a pH value far from its isoelectric point (pH 4-5 for type B gelatin) (11).

Acetone was added as a desolvating agent. The addition of acetone drop by drop caused turbidity of the gelatin solution so that high and low-weight gelatin molecules could separate and indicated the formation of nanoparticles (24). The use of acetone as a desolvating agent is more effective in the synthesis of nanoparticles because the hydrophobic bond in gelatin increases so that the desolvation process is more effective and produced a low polydispersity index (25). The addition of glutaraldehyde as a cross-linking agent has the potential to improve the stability of particles and emulsions (26), beside that, it can also reduce solubility and decrease decomposition speed, and increase mechanical strength because gelatin has low mechanical strength and fast decomposition speed if used in nanoparticle synthesis (27). Glutaraldehyde has an aldehyde group (-CHO) that reacted with the amino group of gelatin, resulting in the formation of an aldimine bond (CH=N). One molecule of glutaraldehyde can bind to two amine groups.

The GNMW solution was then stirred for 3 hours using a magnetic stirrer. The particle size was directly proportional to the length of stirring time. The longer the stirring process, the smaller the particle size that will be produced. The increase in the length of stirring time caused the greater strength of the solvent molecules to contact with gelatin so that more and more particles split into nano-sized particles (28). The nanoparticle solution was then purified by the dialysis method using a dialysis tube soaked in water for 24 hours. The water of dialysis baths was changed every 2 hours. Dialysis tubes served to separate particles with different molecular weights. Larger molecular weights could be retained and smaller molecular weights would be liberated (29). The dialysis tube used had a molecular weight of 12 kDa, while the average relative molecular weight of commercial gelatin is between 20-70 kDa so that gelatin nanoparticles could

be retained by the pores of the dialysis tube and produced a purer nanoparticle solution. The resulting nanoparticles were in the form of suspensions, yellowish-clear in color with a characteristic odor.

5. GNMW characterization

Measurement of particle size and polydispersity index was carried out based on the Dynamic Light Scattering (DLS) method. This method is usually used for the measurement of suspended particles in liquids. The advantage of this method is that it can analyze very small samples of less than 3 µL. DLS works based on the principle of measuring Brownian particle motion, which is the free movement of particles due to friction from the surrounding solvent molecules. The particle size obtained was 200.3 nm. The requirements for the size of the nanoparticles needed in the drug delivery system are 50-300 nm (30). It could be concluded that the particle size met the requirements. The increase in particle size could be affected by the pH value and stirring speed.

The polydispersity index value shows a description of the particle size distribution. The polydispersity index value provides information about the stability of the dispersion system formed for a longer period and the drug release profile (31). The particle size is said to be uniform if the value of the polydispersity index is small. The requirement for the value of the polydispersity index of nanoparticles is 0-1. If the value of the polydispersity index is close to 1, it indicates that the particle size distribution shows high diversity and contains large particles or aggregates that have precipitated. A polydispersity index value of 0-0.5 indicates a uniform particle size distribution (32). The polydispersity index value was 0.288. It could be concluded that the polydispersity index value met the requirements and was monodispersed which indicated that GNMW had a uniform particle size distribution so they tend to be stable.

Zeta potential is a parameter of electric charge between colloidal particles which is influenced by changes in the interface with the dispersing medium, due to the dissociation of functional groups on the particle surface or ionic absorption that appears in the liquid dispersion medium and the occurrence of the solvation effect. Zeta potential was measured using the Electrophoretic Light Scattering (ELS) technique or Laser Doppler Microelectrophoresis. When the suspension is given an electric current, every charged particles will move at a speed and direction by its zeta potential value (32). The zeta potential measurement is said to be eligible if it has a zeta value less than -25 mV and greater than +25 mV because it has high stability and can prevent the formation of particle aggregation on the surface charge (23). The zeta potential value of GNMW was +16.11 mV. The zeta potential was positive because the nanoparticles contain MW extract which made an acidic atmosphere. When the pH is low, the amine group (NH_4^+) in gelatin will react with glutaraldehyde. Positively charged nanoemulsions are more effective in the diffusion process inside the skin because the positively charged particles of the nanoemulsion system can to carry the drug into the skin optimally and then encourage drug penetration through the skin. The level of skin binding with positively charged drugs is higher than with negatively charged drugs because it is known that the skin is negatively charged at neutral pH (34).

FTIR measurement on gelatin nanoparticles of MW extract showed that there was an interaction between gelatin and watermelon mesocarp extract, which was indicated by a shift in the wave number in the O-H group from 3267.57 cm^{-1} for MW extract and 3286.78 cm^{-1} for gelatin to 3377.47 cm^{-1}. The shift of the amide band (C=O), arose from the stretching vibration of the N-H group which was combined with the -OH group through hydrogen bonds. The wave number of the amide group shifted to a lower direction when a cross-linking agent such as glutaraldehyde was added. This was associated with the cleavage of hydrogen bonds to maintain the helical structure of gelatin (35). The result of FTIR is shown in Figure 1.

SEM characterization results showed that the size of all nanoparticles was in nanometers. The particle size measured by SEM was larger than that measured by DLS. This phenomenon could be happened because the intensity of Rayleigh scattering light is proportional to the sixth power of the diameter of nanoparticles. Hence, the signal of larger particles will be easy to record, and the smaller particles will be missed (36). The powder was sticky and piled up, resulting in an irregular shape. This is because the sample did not dry completely and shake well. The result of SEM is shown in Figure 2. The particle size measured by SEM was larger than that measured by DLS. This phenomenon could be happened because the intensity of Rayleigh scattering light is proportional to the sixth power of the diameter of nanoparticles. Hence, the signal of larger particle is easy to be recorded, and the smaller one will be missed.

6. GNMW drying method

The method used in the drying process of GNMW was freeze drying. Freeze drying method is the best method used in the development of pharmaceutical preparations because it is suitable for use in samples that are thermolabile so that it does not damage the compounds contained, produces more attractive colors, and is more stable in quality (no changes in odor, color,

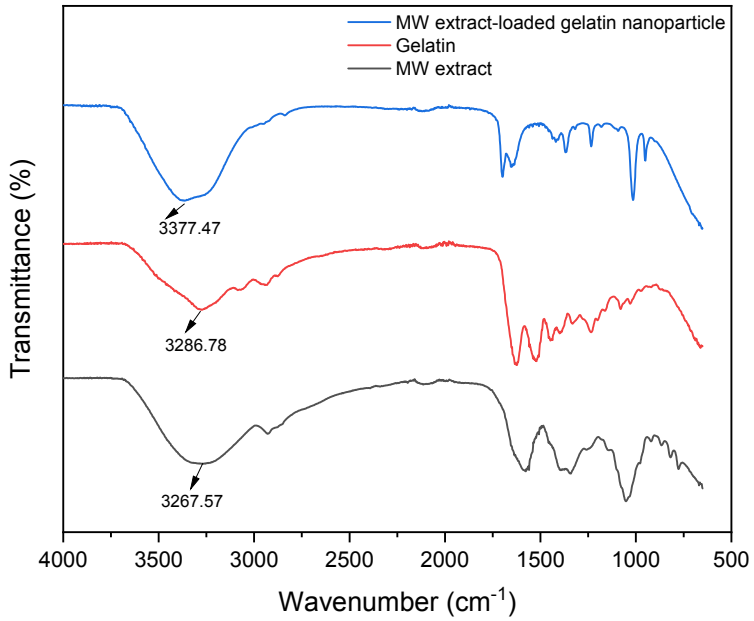
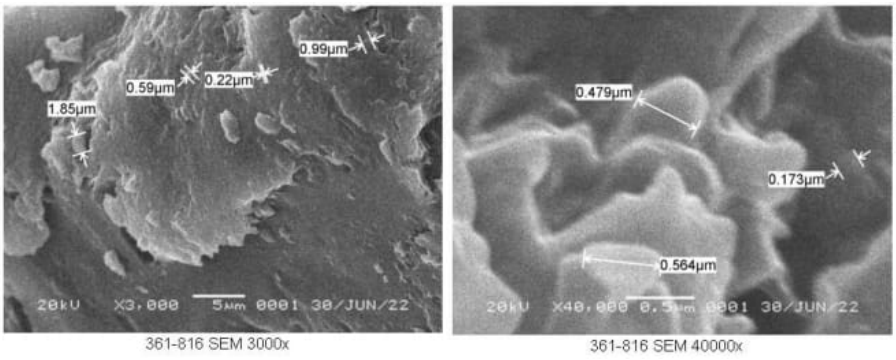


Figure 1. FTIR spectra of MW extract, gelatin, and MW extract-loaded gelatin nanoparticle



(a) (b)

Figure 2. SEM characterization results of GNMW at a magnification of 3,000x (a) and 40,000x (b)

and other organoleptic parameters). A specific amount (100 mL) of GNMW suspension was freeze-dried for 48 hours. GNMW powder was obtained for 0.4108 g. GNMW powder was pale yellow with a characteristic odor. GNMW powder was soluble in water, very slightly soluble in propylene glycol, and practically insoluble in ethanol.

7. Gel preparation

Gel preparation was conducted by developing Carbopol 940 g with 20 times of water at a temperature of 70 °C then allowed to stand for 30 minutes so that Carbopol 40 powder could absorb water and expand optimally. TEA is one of the neutralizing agents that will ionize Carbopol and will create a negative charge on the polymer, resulting in a repulsion force that will cause the formation of an extended three-dimensional structure that looks like a honeycomb formation in the gelling process, so that the gel will have a stable consistency and an increase in viscosity (37). Phenoxyethanol has antimicrobial activity. The concentration of phenoxyethanol that can be used in topical preparations is 0.5% - 0.1% (37). Propylene glycol was used as a humectant that would maintain the water content in the gel preparation so that the gel had good stability. In addition, propylene glycol could increase solubility, so that the active substance would dissolve and penetrate easily into the skin. The maximum concentration of propylene glycol that can be used in topical preparations as a humectant is 15% (38). Sodium metabisulfite was used as an external antioxidant to prevent oxidation in gel preparations. As stated in Maia et al., sodium

Table 3. pH, viscosity, and spread-ability value of GNMW gels

Evaluation parameters	F0	F1	F2	F3	Standard value
pH	7.23 ± 0.03	7.08 ± 0.12	6.96 ± 0.11	6.68 ± 0.07	6.0 – 8.0
Viscosity (cP)	14,673 ± 8.27	15,427 ± 189.03	16,720 ± 131.15	17,073 ± 94.52	3,000 – 50,000
Spread ability (cm)	6.4 ± 0.1	5.8 ± 0.1	5.62 ± 0.08	5.47 ± 0.15	5 – 7

metabisulfite was used as an antioxidant for vitamin C because it possesses limited stability as an antioxidant agent. To assess the vitamin C chemical stability in semisolid cosmetic formulations, several aspects must be regarded like exclusion of the oxygen, protection against the light and temperature, and the utilization of an efficient antioxidant system (39). GNMW gel containing MW extract which is supposed to have antioxidant activity like vitamin C as a positive control, also requires external antioxidants to provide optimal antioxidant activity to the gel preparations. The concentration of sodium metabisulfite that can be used as an antioxidant in pharmaceutical preparations is 0.01%-1% (38).

8. Evaluation of GNMW gel

GNMW gel produced was clear. There was no difference in the results of the organoleptic test on F0, F1, F2, and F3. GNMW gels had a neutral pH of 6,7 - 7,3 so they were safe for use on the skin. The homogeneity test on all gel formulations showed homogeneous results, indicated by the absence of lumps or coarse granules in the preparation. The results obtained met the requirements of the gel homogeneity test. The homogeneity test is related to the therapeutic effectiveness of the preparation. If the preparation is homogeneous, it is ensured that the concentration of the active substance is uniform, so that it will be evenly dispersed on the skin. The viscosity of GNMW gels was 14,580 – 17,180 cP. The value of viscosity increases with the increase in the concentration of the active substance. This is because MW extract contains 21.03% of pectin compound which can be used as a gelling agent (40) and in the process of making watermelon mesocarp extract nanoparticles using gelatin which functions as a thickening agent. The viscosity met the requirements, which are in the range of 3,000 – 50,000 (41). The spread-ability of all gel formulations was in the range of 5.3 – 6.5 and met a good spread-ability standard value. The value of pH, viscosity and spread-ability are shown in Table 3.

9. Irritation test

The irritation test was performed by applying the preparations on the upper forearm of the volunteers. The skin of the upper forearm was chosen because the area has a thin and sensitive horn layer, so the absorption of the sample is quite large. There is a minimal movement in that area, so the attached sample can be in contact with the skin for a long time. The irritation test was carried out by the patch tester method to protect the sample from external influences (42). Based on the results of the GNMW gels irritation test, there was no irritant reaction in all volunteers.

10. Skin moisture test

Skin moisture is a condition that is influenced by the water and oil levels in the skin. If the moisture content is insufficient, it can cause dry skin or xerosis cutis. The water content in the stratum corneum in normal skin is about 10% in the outer layer and about 30% in the deeper layer. A decrease in the water content in the stratum corneum to less than 10% can cause the skin to become scaly, rough, and dry. Normal skin has a moisture content of 30-50% (43).

The results of the moisture test showed that GNMW gel with various concentration of 0% (F0), 0.0114% (F1), 0.0228% (F2), and 0.0342% (F3) could increase skin moisture after 3 hours of use. The moisture content of the panelists dull skin (without applying GNMW gel preparation) had an average value of 26.770% - 32.720%. After the application of GNMW gel preparations of F0, F1, F2, and F3 the moisture content increased from 38.810% - 63.190% (Figure 3.). The increase in skin moisture occurs due to the increased concentration of GNMW added to each formula. Okzelia & Mardiyyah showed that the higher the concentration of MW extract added to the gel preparation, the higher the percentage of skin moisture (44). GNMW also played a role as an antioxidant to prevent the skin from drying out due to UV exposure. MW extract had compounds in the form of citrulline and flavonoids as natural antioxidants. With this ability, MW extract can prevent skin cells from being damaged, including skin dryness so that the skin was kept moist.

11. Antioxidant activity assay

The principle of measuring antioxidant activity with the DPPH method is that there is a change in the intensity

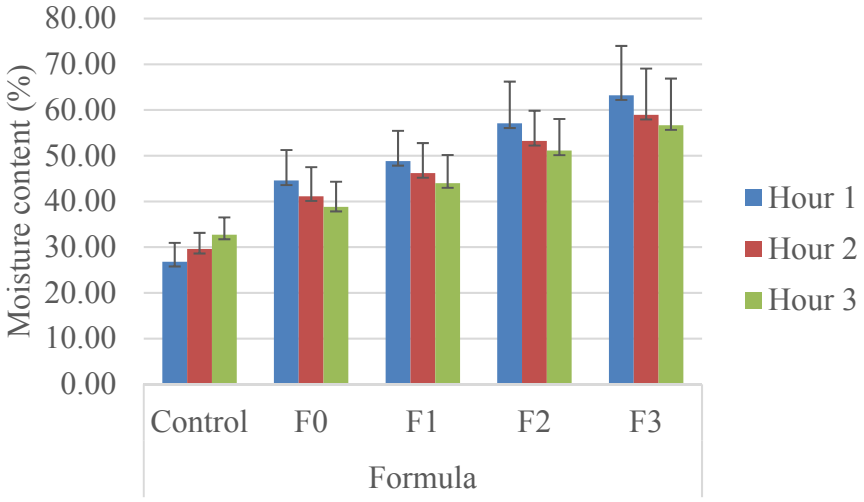


Figure 3. Profile of moisture content of GNMW gels toward volunteers skin

Table 4. Antioxidant activity test result using the DPPH method

Sample	IC_{50} value (µg/mL) ± SD
Vitamin C	7,89± 0,22
MW extract	110,90 ± 1,74
GNMW	114,16 ± 2,15
GNMW gels:	
F0 (basis)	161,62 ± 0,88
F1	149,52 ± 0,93
F2	138,44 ± 3,89
F3	127,10 ± 0,94

of the purple color from DPPH to pale purple to yellow. This happens because there is a reaction of the DPPH molecule with the hydrogen atom given by the compound molecule in the sample so that it is reduced to DPPH-H which forms the compound 2,2-diphenyl-l-picrylhydrazyl (45). The maximum wavelength of DPPH was scanned using a UV-Vis spectrophotometer. The results showed that the maximum wavelength of 100 ppm DPPH solution was 517.1417 with an absorbance of 0.757. The decrease in DPPH absorbance was measured against the control absorbance (DPPH in ethanol in a ratio of 1:3, without adding a sample). Qualitative analysis of the decrease in absorbance was indicated by a change in the color of the DPPH solution from pale purple to yellow. The intensity of the color of the solution is directly proportional to the increase in the concentration of the sample. A compound is categorized to have very active antioxidant activity if the IC_{50} value is < 50 µg/mL, active is 50-100 µg/mL, moderate is 101-250 µg/mL, weak is 250-500 µg/mL, and inactive is >500 µg/mL (46). The result of the antioxidant activity assay of MW, GNMW, and GNMW gel preparations and vitamin C is shown in Table 4.

Vitamin C as positive control had a very active antioxidant activity because it can donate hydrogen atoms and form relatively stable ascorbyl free radicals. MW extract had moderate antioxidant activity because it contained flavonoid compounds that function as antioxidants. In flavonoid compounds, there are free hydroxyl groups that have an activity to capture radicals and function to ward off new free radicals by breaking the chain reaction and turning them into more stable compounds. The increase in the IC_{50} level indicated a decrease in the antioxidant activity of GNMW and GNMW gels, which could be due to the mixing the active substance in the form of an extract containing antioxidants with other additives in the produced nanoparticle. In addition, the results showed that as the concentration of antioxidant gel increased, IC_{50} values decreased, indicating a significant effect of increasing concentration on antioxidant activity ($p<0.05$). The comparison of antioxidant activity is shown in Figure 4.

Conclusion

Antioxidant moisturizing gels had been formulated using GNMW extract in various concentration of 0.0114% (F1), 0.0228% (F2), and 0.0342% (F3) which met evaluation parameters criteria such as pH, viscosity,

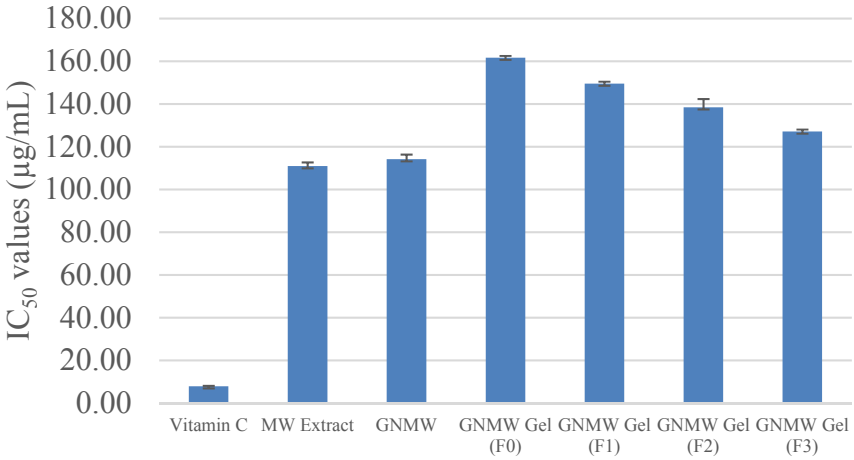


Figure 4. Comparative profile of IC_{50} value of vitamin C, MW extract, GNMW, and GNMW gels

spread-ability, did not irritate the skin, had an IC_{50} value in the range of 127,10 ± 0,94 - 149,52 ± 0,93 µg/mL and could increase 38,81% - 63,19% skin moisture content.

The antioxidant activity of developed GNMW moisturizing gel in this study categorized as medium. The concentration of GNMW extract could be further increased to obtain antioxidant activity with a strong or very strong category.

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