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Research Article

FORMULATION AND ANTIOXIDANT ACTIVITY OF NANO GEL ETHANOL EXTRACT OF KEPOK BANANA PEEL (*MUSA X PARADISIACA* L.)

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ABSTRACT

Kepok banana peel (*Musa x paradisiaca* L.) contains flavonoids that act as antioxidants. The antioxidant activity of the ethanol extract of Kepok banana peel can be used as an active ingredient of nano gel. Nano-sized preparations can increase the efficiency of the delivery of active ingredients with lesser side effects and also decrease the stratum corneum to make it easier to penetrate. The objectives of this study were to determine whether the ethanol extract of Kepok banana peel (*Musa x paradisiaca* L.) can be formulated as nano gel and to measure its antioxidant activity. The method of nano gel preparation was stirring technique by using a magnetic stirrer and a sonicator. The antioxidant activity was measured using DPPH method. The results showed that the ethanol extract of kepok banana peel could be formulated as nano gel with size F0 = 161.9 nm; F1 = 171.3 nm; F2 = 165.6 nm; F3 = 163.9 nm which fulfilled the nano gel size requirement (20-200 nm) and the potential zeta value F0 = -43.4 mV; F1 = -43.7 mV; F2 = -46.9 mV; F3 = -47.0 mV fulfilled the requirements (smaller than -30 mV and greater than +30 mV). The antioxidant activity showed that the IC_{50} of ethanol extract of was stronger than IC_{50} of 1% nano gel (198.0279 µg/ml : 282.8933 µg/ml).

Keywords: Banana peel; Nano gel; Antioxidant activity.

INTRODUCTION

The skin is an organ that covers the entire human body and is always seen first and is considered as one of the elements of beauty. So it is necessary to maintain its condition from the dangers of the surrounding environment such as air pollution, sunlight, and other chemicals. The skin must always be treated so that its appearance remains beautiful and healthy and radiates freshness to those who view it[1]. With increasing age, the skin will experience an aging process that looks dull and wrinkled, the skin becomes old quickly and black spots appeared[2]. To help whiten the skin, there are several ways of handling that is by using antioxidants. Antioxidants are used to protect the skin from oxidative damage so that it can prevent premature aging[3].

Antioxidants are substances that protect from free radical attacked. Free radicals are wild compounds that are very dangerous because they will trigger a chain reaction. Some antioxidants can be produced from natural products, such as herbs, vegetables, and fruit. One of them is banana peel which has high antioxidant activity compared to the flesh of the fruit. Based on previous research[4] antioxidant compounds found in banana peel extract, one of which is epicatechin which is a class of flavonoid compounds. Based on the results obtained, the antioxidant activity of Kepok banana peel extract was 95.14%, so that the banana peel extract could withstand the DPPH radical of 95.14%.

The utilization of antioxidant effects on preparations aimed at the skin is better when formulated in the form of topical cosmetic preparations compared to oral[5]. Currently, antioxidants have been circulating among others in the form of a gel, cream, serum, and tablet preparations. The stratum corneum has a brick-like structure that makes it difficult for drugs to penetrate through skin tissue[6]. One way to overcome the problem of penetration is to make the drug particles as small as possible to the size of nanometers which is 20-200 nm. In this study the design of nanoparticle preparations was carried out as a drug delivery system, one of the dosage forms was nano gel. Nano gel isnano-sized hydrogel systems which are highly interrelated involve polymer systems that are both polymerized or monomers[7]. The previous study has researched natural nano gel from Mentha piperata extract with a size of 224.8 nm-557.6 nm[8]. The advantages of using nanoparticles as a drug delivery system include particle size and surface characteristics of nanoparticles can be easily manipulated according to treatment targets, nanoparticles regulate and prolong drug release during drug transforcement to the target, and nanoparticle systems can be applied to various treatment targets because nanoparticles enter into the circulatory system and under the blood to target treatment[9]. Based on the description above, the researchers are interested in formulating ethanol extract kepok banana peel (*Musa x paradisiaca* L.)in the nano gel preparation formula, as well as testing the antioxidant power of the preparation. To produce quality nano gel preparations, it is necessary to make an experiment to make the right formulation.

MATERIALS AND METHODS

Sample Preparation

Kepok banana peel (*Musa x paradisiaca* L.) was used in this study which was taken in the village of Padang Mutung, Kampar District, Kampar District, Riau. Kepok banana peel was washed with water and chopped to make it easy in the drying process. The dried sample was blended to make powders.

Extract Preparation and Chromatography Analysis

The extract was made by maceration using 70% ethanol. A total of 400 g of dried powder of Kepok banana peel was put in a maceration container using 1 L 70% ethanol until the simplicia was completely submerged, then concentrated using a rotary evaporator at 60 C°until a thick extract was obtained. The compositions of extract were analyzed by using chromatography technique with toluene: acetone: formic acid (6: 6: 1) as mobile phase and observed under UV light 254 nm. The spots and the Rf were calculated.

Antioxidant Activity Test
Preparation of DPPH Solution 30 µg / mL

10 mg DPPH was diluted with methanol in a 100 mL volumetric flask to the boundary mark then shaked until homogeneous and obtained a solution with a concentration of 100 µg / mL. then diluted by piping 15 mL DPPH solution in a concentration of 100µg / mL put into a 50 mL volumetric flask sufficient with methanol solvents to mark the limit to obtain a DPPH solution with a concentration of 30 µg / mL.

Preparation of Blank Solution and Maximum Wavelength Optimization of DPPH

Pipette 3.8 mL of DPPH (30 µg / mL) solution into the test tube. Then methanol was added as much as 0.2 mL and homogenized and the mouth of the tube covered with aluminum foil. Then incubated in a dark room for 30 minutes. Determine the absorption spectrum using a UV-Vis spectrophotometer at a wavelength of 400-800 nm and determine the maximum wavelength.

Preparation of Gallic Acid Standard Solution

Gallic acid weighed as much as 10 mg, dissolved with enough methanol, put in a conical flask, and then added methanol to 100 mL obtained a mother liquor with a concentration (100 µg / mL). Furthermore, a series of concentrations of 5 µg / mL, 10 µg / mL, 15 µg / mL, 20 µg / mL and 25 µg / mL were made using a pipette of 0.5; 1; 1.5; 2; and 2.5 mLfrom a solution of a concentration of 100 µg / mL put in a 10 mL volumetric flask sufficient to mark the limit with methanol. Nextpipette each as much as 0.2 mL of the gallic acid solution and put into vials, then added 3.8 mL of DPPH solution (30 µg / mL). The mixture is homogenized and left for 30 minutes in a dark place. The absorption spectrum was determined using a UV-Vis spectrophotometer at the maximum wavelength of DPPH (515.50 nm).

Antioxidant Activity Study

Weighed as much as 100 mg extract was dissolved in 100 mL methanol (1000 µg / mL). Then dilution is carried out to obtain a serial solution concentration of 50 µg / mL, 100 µg / mL, 150 µg / mL, 200 µg / mL, 250 µg / mL by means of piping each 0.5; 1; 1.5; 2 and 2.5 mL of a solution of 1000 µg / mL concentration were added in a 10 mL volumetric flask sufficiently to the mark with methanol. Each solution was piped as much as 0.2 mL mixed with 3.8 mL of DPPH solution (30 µg / mL), shaken, and allowed to stand for 30 minutes at room temperature in a dark place. Then each mixture was measured at the maximum wavelength of DPPH (515.50 nm) with a UV-Vis spectrophotometer. The antioxidant activity of the sample is determined by the amount of DPPH radical uptake resistance through the calculation of the percentage of DPPH absorption inhibition. The percent inhibition results are substituted in linear equations. The result of the substitution of percent inhibition is then interpreted as IC_{50}. IC_{50} is defined as the number of antioxidants needed to reduce the initial DPPH concentration by 50%.

Nano Gel Ethanol Extract preparation

In this study, the formulas used can be seen in Table 1. Each formula was made for 50 g.

Table 1. Nano Gel Formula

Material	F0	F1	F2	F3
Ethanol Extract Kepok Banana Skin	-	1	1	1
Carbopol 940	1	1	1	1
Glycerin	5	5	5	5
Propylene glycol	10	10	10	10
TEA	1	1	1	1
Methyl Paraben	0.18	0.18	0.18	0.18
Propyl Paraben	0.02	0.02	0.02	0.02
Aquadest up to Speed (rpm) Time (hours)	50 400 1	50 600 1	50 800 1	50 1000 1

The manufacture of nano gel based on a 940 carbopoly base begins with weighing the ingredients according to Table 1. Carbopol 940 sprinkled on hot water as much as 20 mLAfter expanding, stirring is carried out continuously with a magnetic stirrer until it is completely dispersed, then add TEA to form a gel mass. Furthermore, methylparaben and propylparaben were dissolved with hot water and then put into a gel base. After that enter the ethanol extract of Kepok banana peel which has been mixed with glycerin and propylene glycol until homogeneous. Then, when stirring, add the remaining distilled water little by little until it is homogeneous. After that, sonication was carried out by inserting the preparation into the ultrasound device for 1 hour.

Evaluation of Nano Gel
Organoleptic Test

An organoleptic test is done to see the physical appearance of the preparation by observing the shape, color, and odor of the preparation that has been made[10].

Homogeneity Test

Homogeneity testing is done using a sample of nano gel formula is placed on the slide, observed the composition of coarse particles or inhomogeneity, then recorded. The preparation must show a homogeneous arrangement and no coarse grains are seen[11].

pH test

Before measuring the pH meter, it is calibrated first. The pH measurement is done by dipping the electrodes into the nano gel ethanol extract of Kepok banana peel, as much as 1 g of the preparation dissolved in water with a volume of 10 mL, then measuring the pH using a pH-meter wait until the screen on the pH meter shows a stable number. The pH of the nano gel preparation must be by the pH of the skin which is 4.5–8.0[12].

Stability Test

The physical stability test in this study used the cycling test method. 1 gram of the preparation is stored at 4 ° C for 24 hours, after that, the preparation is put in the oven at 40 ° C for 24 hours. The treatment is carried out in one cycle. Observe the occurrence of physical changes such as color, odor, and shape of the nano gel preparation.

Scattering Test

The spread test is carried out to determine the speed of spread of nano gel on the skin when applied to the skin. A total of 1 gram of nano gel is carefully placed on a 20x20 cm glass. Next covered with another glass used ballast on it until the weight reaches 100 g and measured its diameter after 1 minute. Scattering power requirements are 5-7 cm[13].

Irritation Test

The irritation test was carried out on 12 volunteers. Testing is done by open patch test (Patch test). An open patch test is carried out by applying preparations to the inner forearm made at the location of the attachment with a certain finished area (2.5 x 2.5), left open, and observing what happens. A positive irritation reaction is characterized by the presence of redness, itching, or swelling in the skin of the inner forearm that is treated[14].

Droplet size test

Analysis of particle grain distribution using the Particle Size Analyzer (PSA), which works based on the principle of Dynamic Light Scattering. This method uses a dispersing medium to disperse the sample. Distilled water dispersing media. Samples were measured three times until two data were obtained that had a difference of less than 20 nm[15].

Potential Zeta

Zeta potential is a measure of the surface charge of particles dispersed in a dispersing medium. Nanoparticles with a potential zeta value of less than -30 mV and greater than +30 mV because the higher the zeta potential will further prevent flocculation, ie the event of combining colloids from small to large which has higher stability[16].

Test the antioxidant power of the formulation

Weighed as much as 500 mg extract was dissolved in 100 mL methanol pro analysis (1000 µg / mL). Then dilution was carried out to obtain a series solution of 150 µg / mL, 200 µg / mL, 250 µg / mL, 300 µg / mL, 350 µg / mL by piping 1.5 each; 2.0; 2.5; 3.0 and 3.5 mL of a concentration of 1000 µg / mL were added to a 10 mL volumetric flask to the limit markings with methanol pro analysis. Each solution was piped as much as 0.2 mL mixed with 3.8 mL of DPPH solution (30 µg / mL). Each mixture is shaken and allowed to stand for 30 minutes at room temperature in a dark place. Then the absorption of each mixture was measured at a wavelength of 400-800 nm with a UV-Vis spectrophotometer. The antioxidant activity of the sample is determined by the amount of DPPH radical absorbance inhibition through the calculation of the percentage of DPPH absorbance inhibition.

RESULTS AND DISCUSSION

Kepok banana skin sample (*Musa x Paradisiaca* L.) used as much as 5 kg. Simplisia obtained from drying is 400 g.

Characteristics of Simplisia

The results of the simplicia characteristic test can be seen in Table 2. The extraction process of Kepok banana peel is done by maceration method. Percentage of yield obtained from methanol extract of Kepok banana peel is as higher as 13.36571%.

Table 2. Simplisia Characteristics Test Results

Testing	% content
Dry shrinkage	0.6743
Total ash content	2.7740
Acid insoluble ash content	1.0125
Water-soluble	17.968
Soluble in ethanol	5.1157

Extraction of extracts was carried out on the ethanol extract of Kepok banana peels can be seen in Table 3 below:

Table 3. Testing Results for Specific Characteristics of Ethanol Extract in Kepok Banana Skin

Testing	% Content
Dry shrinkage	0.3613
Specific Weight	1.2641g / mL
Water content	8.3666
Total ash content	0.6405
Water-soluble	27.6202
Soluble in ethanol	21.4207

Chemical Extracts Test

Determination of identity compounds qualitatively by using the TLC method (thin layer chromatography) In this study using ethanol extracts of Kepok banana peel (*Musa x paradisiaca* L.) and the standard used was quercetin. The stationary phase used is GF254 silica gel and the mobile phase used is toluene: acetone: formic acid (6: 6: 1). The extracted sample produced 1 blotch with an Rf value of 0.70 and in the quercetin standard also obtained an Rf value of 0.68. The same Rf value proves that the ethanol extract of Kepok banana peels has the same content as the standard.

Table 4. Results of Chemical Content of Ethanol Extract in Kepok Banana Skin

Testing	Results
Alkaloids	-
Flavonoids	+
Saponin	-
Tannins / polyphenols	+

Antioxidant Activity Test for Ethanol Extract Kepok Banana Skin

Antioxidant testing was carried out quantitatively using DPPH and UV-Vis spectrophotometers. The principle of measuring antioxidant activity by the DPPH method is the change in intensity of the color purple to yellow which occurs because of

the reaction of the DPPH molecule with hydrogen atoms released by the compound molecules in the extract.

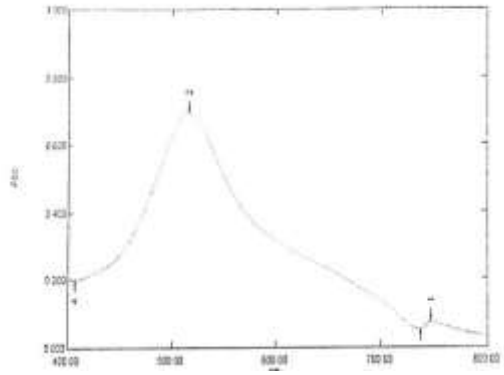

Figure 1. Maximum DPPH wavelength

Free radical scavenging activity is expressed by IC_{50} value which is defined as the concentration of the sample needed to declare inhibition of 50% which means that compounds at that concentration can inhibit free radicals by 50%. IC_{50} values were calculated using a regression equation between the percentage of free radical inhibition and sample concentration.

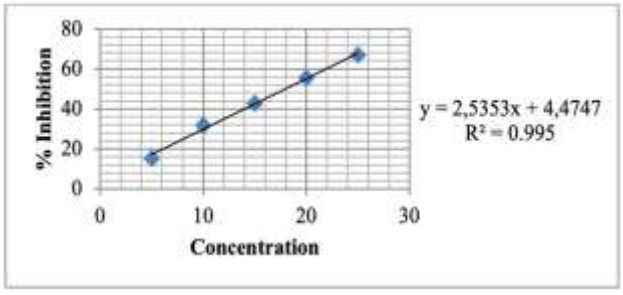

Figure 2. IC_{50} determination curves of several gallic acid standard solutions

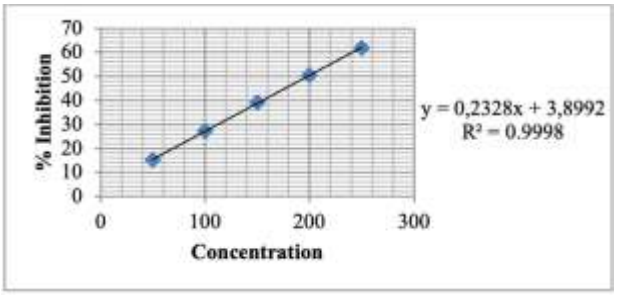

Figure 3. IC_{50} determination curve of extract solutionethanol kepok banana peel

Testing the antioxidant activity of the ethanol extract of Kepok banana peel DPPH method begins with the determination of the maximum wavelength of DPPH with a concentration of 30µg / mL, obtained a maximum wavelength of DPPH of 515.50 nm with the absorbance of 0.695

Furthermore, the measurement by DPPH free radical reduction method is carried out at that wavelength. The results of determining the maximum wavelength of DPPH absorption is 30 µg / mL the ethanol extract of Kepok banana peel yields IC_{50} is equal to198.0279µg / mL whereas, in the gallic acid standard solution, the IC_{50} value is equal to17.9565µg / mL.

Evaluation of preparations

Organoleptic test results of nano gel ethanol extract of Kepok banana peel, namely in F0, F1, F2, and F3 in the form of a gel, F0 white, F1, F2, F3 are yellow, F0, F1, F2, and F3 smell special. Homogeneity test results carried out that the four nano gel preparations are homogeneous and no coarse grains were found. This means nano gel ethanol extract Kepok banana peel meets the requirements of good nano gel that is homogeneous. The results of the pH evaluation of nano gel ethanol extract from Kepok banana peels, namely F0, F1, F2, F3, were obtained as follows: 6.95; 5.87; 5.76; 5.84all preparations are categorized as good for the skin because they meet the skin's pH requirements, namely 4.5-6.5. The more acidic the pH value can irritate the skin and the more alkaline the pH value can make the skin scaly or rough. The results of the stability test showed that the nano gel preparation from the ethanol extract of Kepok banana peel did not undergo separation and physical changes. This shows that there is no decomposition of the active substance due to temperature changes.

The spread test is carried out to ensure the equal distribution of the gel when applied or applied to the skin. The range of good gel dispersion ranges from 5-7 cm[17]. The four formulas F0, F1, F2, and F3 respectively produce an average spread test of 5.3 cm; 5.4 cm; 5.6 cm, and 5.9 cm. The spread test of the four formulas meets the requirements. Skin irritation test is done to prevent the occurrence of side effects on the skin. From the results of irritation tests carried out on the four preparations of ethanol extract of Kepok banana peels, it was found that it did not cause any reaction either redness, itching, or burning. So nano ethanol extract of Kepok banana peel is safe to use as a topical preparation.

The resulting nano gel droplet size tests had particle sizes for F0 of 161.9 nm, F1 of 171.3 nm, F2 of 165.6 nm, and F3 of 163.9 nm. From the results of the PSA test, it can be seen that the particle sizes of F0, F1, F2, and F3 range between 20-200 nm. Each formula has a different size, for smaller particle sizes found in F0 that is without extract with a stirring speed of 400 rpm, while smaller particle sizes using extracts are found in F3 with a stirring speed of 1000 rpm. So it can be concluded that the particle size of each formula has been formed nano preparations and as expected. And for the speed of stirring and the addition of the ethanol extract of Kepok banana peel can affect the particle size of the nano gel formula.

Determination of zeta potential of nano gel is measured to improve the stability of nano gel. Interaction between particles has an important role in the stability of a nano gel. Zeta potential is a measure of the surface charge of particles dispersed in a dispersing medium as well as a measure of repulsion between particles. Ideally, the zeta charge of the particle potential should be higher than the dispersing medium to prevent aggregation. Nanoparticles with a potential zeta value of less than -30 mV and greater than +30 mV have higher stability[11]. The potential zeta results were obtained, for F0 of -43.4 mV, F1 of -43.7 mV, F2 of -46.9 mV, and F3 of -47.0 mV so that it meets the requirements of a potential zeta value that is smaller than -30 mV and greater than +30 mV. The bigger and farther the surface charge is, the less repulsive energy the greater the tendency to rejoin is also getting smaller. Negative values because the polymer used can cover the surface of the nano gel, this causes the mobility of particles will be reduced and between particles will not combine to form aggregates.

Table 5. Results of Nanoparticle and Zeta Potential Measurement of Nano Gel Ethanol Extract in Kepok Banana Skin

No	Sample name	Parameter	Test results
1	F0	Nanoparticles (nm) Potential Zeta (mV)	161.9 nm -43.4 mV
2	F1		171.3 nm -43.7 mV
3	F2		165.6 nm -46.9 mV
4	F3		163.9 nm -47.0 mV

Antioxidation test on nano gel ethanol extract of Kepok banana peel with a concentration of 1% (F1) resulted in IC_{50} of 282.8933 µg / ml, at the level of strength of antioxidant intensity that is very strong <50, strong 50-100, moderate 100-250, weak 250-500, inactive> 500.

CONCLUSION

Based on research that has been done about the formulation and testing of nano gel ethanol extract preparation activities of Kepok banana peel (*Musa x paradisiaca* L.), the following conclusions can be drawn: The ethanol extract of Kepok banana peel (*Musa x paradisiaca* L.) can be formulated in nano gel preparations. The four formulas produced different particle sizes, namely for F0 of 161.9 nm, F1 of 171.3 nm, F2 of 165.6 nm, and for F3 of 163.9 nm. The particle size of each formula has been formed nano preparations and as expected, that is 20-200 nm. Nano gel of ethanol extract of Kepok banana peel has the antioxidant activity of 282.8933 µg / ml ie weak.

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