

# Formulation and Antioxidant Activity of Serum Gel of Ethyl Acetate Fraction From *Musa x paradisiaca* L.

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## ABSTRACT

One of the plants that had potential as a natural antioxidant was raja banana skin. The fraction of ethyl acetate of raja banana skin (*Musa x paradisiaca* L.) had antioxidant activity (IC<sub>50</sub>) with value 50.25 ppm. The fraction of ethyl acetate of raja banana skin will be formulated into serum gel. This study aims to determine the physical characteristics, physical stability and free radical scavenging of serum gel of ethyl acetate fraction of raja banana skin. Concentraions were used in formulation: 0.08 gram (F1); 0.16 gram (F2), 0.24 grams (F3). The raja banana skin was extracted using 96% ethanol by maceration method then be fractionated by using ethyl acetate. Serum gel was formulated by using carbopol as a gelling agent. It was tested for physical properties and physical stability for 28 days. Free radical scavenging activity was tested by DPPH method. Vitamin c as positive control. The results showed that the value of pH, viscosity, spreadability serum gel fraction raja banana skin fulfil requirements. Serum gel fraction of ethyl acetate was stable in 6 cycle because there is no significant change in serum gel, was seen by value of pH, viscosity, or spreadability. The IC<sub>50</sub> value of serum gel fraction of ethyl acetate raja banana skin respectively: 87.947 µg/ml (F1); 84,297 µg/ml (F2); 71,257 µg/ml (F3). The IC<sub>50</sub> value of positive control and negative control were 55,595 µg/ml; and 205,699 µg/ml. Statistical tests using one-way ANOVA showed that the activity of free radical catching activity of the three formulas was weaker than the positive control and fraction control used. F3 was best formulation with high antioxidant activity.

**Keywords:** raja banana skin, serum gel, physical stability, antioxidant

## 1. INTRODUCTION

Free radicals are reactive oxygen contains one or more unpaired electrons in outer orbitals. These compounds are very damaging and can attack protein, carbohydrates, fats, and DNA continuously. Antioxidants are compounds that can counteract the negative effects of free radicals with a mechanism to donate one or more electrons to free radicals [1]. Many antioxidant compounds are found in plants, both in flowers, leaves, and fruit. Plants contain bioactive compounds such as Flavonoids, Alkaloids and Terpenoids are potential raw materials that can be used as natural antioxidants.

One of the plants that have the potential to be natural antioxidant is Raja Banana skin. Banana skin is very rarely used because the skin is only a waste. Banana skin contains many compounds that can function as natural antioxidants.

Flavonoid compounds in the banana skin act as antioxidants. Flavonoid compounds that act as antioxidants in banana skin are isoflavones [2]. Banana skin contains tannin, catechin, gallicocatechin, and epicatechin can act as antioxidants [2]. The ethyl acetate fraction from Raja Banana skin extract had an antioxidant activity with an IC<sub>50</sub> value of 77,068 ppm [3]. In another research, antioxidant activity in banana skin was 73,89 % and the pulp was 66,45% in concentration 0,002 ppm.

Based on the above, The fractionation of banana skin can be formulated as an antioxidant serum gel preparation. Serum gel have the advantage that it can provide a more comfortable effect and easier to spread on the surface of the skin because of its low viscosity and semi-transparent shape. Antioxidant testing used free radical scavenging method by using the DPPH (2,2-diphenyl-1-picrylhydrazyl).

**Table I.** Formulation Of Serum Gel Ethyl Acetate Of Fraction Banana Skin

Material	Concentration %				
	F1	F 2	F3	KN	KP
Ethyl acetate of fraction banana skin	0,08	0,16	0,24	-	Vit C
Carbopol	1,5	1,5	1,5	1,5	1,5
NaOH	0,3	0,3	0,3	0,3	0,3
Glycerin	10	10	10	10	10
Sodium metabisulfit	0,5	0,5	0,5	0,5	0,5
Methyl paraben	0,25	0,25	0,25	0,25	0,25
Etanol	3	3	3	3	3
Aqua deion	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

K N : Negative control group

F1 : formulation with a concentration of 0.08 gram of ethyl acetate fraction of banana skin

F2 : formulation with a concentration of 0.16 gram of ethyl acetate fraction of banana skin

F3 : formulation with a concentration of 0.24 gram of ethyl acetate fraction of banana skin

## 2. MATERIAL & METHOD

### 2.1 Material

Banana skin obtained from Rempoa village, Baturraden subdistrict, Purwokerto regency. The other material: vitamin C, sodium bicarbonate, carbopol, NaOH, glycerin, sodium metabisulfite, methylparaben, 96% ethanol, DPPH, methanol pro analysis, rotary evaporator (IKA) and viscometer brookfield field LV.

### 2.2 Method

#### 2.2.1 Plant Determination

The Banana skin was determined at the Environmental Laboratory, Faculty of Biology, Universitas Jendral Soedirman,

#### 2.2.2. Extraction and Fractionation of banana skin

Banana skins were washed and dried in a dryer cabinet. The dry simplisia was powdered by a blender and sieved with a 40 mesh sieve. The extraction of dried simplisia used the maceration method. 500 grams of dried simplisia was extracted with 5000 ml of ethanol 96%. The liquid extract obtained was concentrated using a vacuum rotary evaporator. Then, The extract was fractionated with ethyl acetate.

#### 2.2.3 Test for Flavonoid Content and Tannins on Ethyl Acetate of Fraction Banana Skin

##### 2.2.3.1 Flavonoid Test

Wilstatter test

The fraction was added 2-4 drops of concentrated HCl and 2-3 small pieces of Mg metal. Changes occur are observed from dark yellow to orange [4].

Bate-Smith test

The fraction was added with concentrated HCl then heated for 15 minutes over a water bath. The positive reaction if it gives a red color [5].

##### 2.2.3.2 Tannin Test

0.1 grams of ethyl acetate fraction of Banana skin was added to 10 ml of hot water, boiled for 5 minutes, and filtered. Part of the filtrate was added with a 1% FeCl<sub>3</sub> solution. The Positive result was shown by the formation of a blackish green color [6].

#### 2.2.4 Formulation of serum gel with ethyl acetate of fraction banana skin.

The design of the formula for serum gel ethyl acetate fraction of banana skin could be seen in table 1. The carbopol was dispersed in water with a homogenizer with a used speed of 1200 rpm and methylparaben was dissolved in ethanol. It was

mixed with glycerin. Sodium metabisulfite and NaOH were dissolved in aquadeion respectively. It was put into the carbopol dispersion then the NaOH solution was added last. Furthermore, the homogenization process used homogenizer with a speed of around 1200 rpm and increased to 1500 rpm.

#### 2.2.5 Evaluation of Serum Gel Ethyl acetate fraction of banana skin

##### 2.2.5.1 Organoleptic Observations

Organoleptic observations include observations of changes in odor, shape, color, homogeneity, and texture.

##### 2.2.5.2 Measurement of pH

The pH test of the formula was carried out using a pH stick. This is done by dipping the pH stick into the preparation.

##### 2.2.5.3 Viscosity Measurement

Viscosity measurements were carried out with viscometer brookfield LV on spindle 64 with use a rotation speed of 50 rpm.

##### 2.2.5.4 Spreadability Test

0.5 grams of serum gel is placed on a round glass, then covered with another glass that has been weighed. Left for 1 minute then measured the diameter of the spread, after 1 minute added a load of 50 grams. Left for 1 minute then measured again the diameter of the spread.

#### 2.2.6 Cycling Test Method

The serum gel was stored at 4°C for 24 hours, then transferred to an oven at 40 ± 2 ° C for 24 hours (one cycle). The test was carried out in 6 cycles and then carried out organoleptic observations (changes in color, odor, and homogeneity [6].

#### 2.2.7 Antioxidant Activity Test

##### 2.2.7.1 Determination of Wavelength

0.01 gram of DPPH was dissolved in 250 ml of methanol (0.004%). 1 ml 0.004% DPPH solution added with 4 ml methanol, shake until homogeneous and measured absorption in the wavelength range 400-800 nm [7].

##### 2.2.7.2 Determination of the Operating Time for DPPH Solution

1 ml of 0.004% DPPH solution added with 4 ml of methanol. Then it was shaken then the absorption observed at minutes 5, 10, 15, 20, 25, 30. It could determine the operating time.

2.2.7.3 Blank Solution

1 ml of 0.004% DPPH solution is added to 10 ml of methanol then homogenized by shaking.

2.2.7.4 Preparation of banana skin fraction concentration series

The ethyl acetate fraction of banana skin was weighed as much as 0.1 gram then dissolved in 100 methanol. The ethyl acetate fraction of plantain peel was made into a concentration series of 25, 50, 75, 100, and 125 ppm.

2.2.7.5 Preparation of a serum gel test solution concentration series

0.01 gram of each serum gel formula reconstituted with 10 ml of methanol. Concentration series were made 25, 50, 75, 100, and 125 ppm.

2.2.7.6 The antioxidant activity test of the ethyl acetate fraction of banana skin.

Antioxidant activity testing was carried out on all concentration series, both fractions, preparations, and vitamin C (positive control) that had been prepared. 2 ml sample added 2 ml of DPPH solution 0.004% then read at a wavelength of maximum absorbance. The percentage of inhibition of the ethyl acetate fraction of banana skin was calculated against DPPH free radicals.

Antioxidant activity can be determined by the following equation:

$$\% \text{Inhibition} = \frac{A_{\text{DPPH}} - A_{\text{Test Solution}}}{A_{\text{DPPH}}} \times 100$$

% inhibition = percentage inhibition of antioxidants

A control = absorbance of DPPH

A Test Solution = absorbance of the test solution

2.2.7.7 IC50 measurement

Furthermore, the data obtained are processed use a linear equation of the form  $y = bx + a$  between the concentration of the serum gel test solution (x) and the percentage of free radical scavenger activity (y). This linear regression equation is used to determine concentration of the serum gel test solution and vitamin C could inhibit 50% of the absorbance of the DPPH.

2.2.8 Results Analysis

Organoleptic, homogeneity, spreadability and pH values were analyzed by descriptive. Meanwhile, the results of the viscosity test, stability test, and IC<sub>50</sub> were analyzed using the One Way Variant Analysis (ANOVA) method with a confidence level of 95%. Free radical scavenging activity data of serum gel were calculated with an IC<sub>50</sub> value and analyzed by a one way ANOVA test with a confidence level of 95%.

**3. RESULT & DISCUSSION**

3.3.1 Plant Determination

The results of plant determination indicated that the plants used in this research were raja banana skin (*Musa x paradisiaca* L.) from the Musaceae family .

3.3.2 Extraction and Fractionation of banana skin

The extraction of banana skin was carried out using the maceration method. It was because the process does not use heat so that it can keep the thermolabile components like flavonoids [9]. In this method, ethanol 96% was used as a solvent to extract flavonoids and tannins. Ethanol has been known as a good solvent for polyphenol extraction such as flavonoid and safe for human consumption [10].

The viscous extract obtained from the extraction of banana skin was 72.29 grams and the randemen value was 14.458%. The extract obtained is brownish yellow, thick, and had a distinctive smell of banana skin. The next process was fractionation. The ethyl acetate fraction of thick banana skin obtained was 6.4 grams and the randemen value was 12.8%. From these results, it could be concluded that the randemen value in the process extraction and fractionation was small. The reason for small randemen value was carried out in stages fractionation and extraction so that many active compounds had been found in the previous solvent.

3.3.3 Identification of Flavonoids and Tannins in Ethyl Acetat of Fraction of Banana Skin

3.3.3.1 Flavonoid Test

Wilstatter Test

The results obtained were that the ethyl acetate fraction of plantain fruit peels changed color from yellow to reddish-orange (fig 1). These changes color indicate that the skin of the banana contains flavonoids. The addition of HCl in the wilstatter test aims to hydrolyze flavonoid to flavonoid aglycones by hydrolyzing o-glycosyls. This hydrolyzed glycosyl will be replaced with H + atoms from acids which have strong electronegativity properties. Mg powder was added in the solution produces a complex compound. It was marked by color change become red color. This magnesium ion will bind to the flavonoid compounds present in the fraction so that a red solution appears [9].



**Figure 1** Wilstatter Test Result

**Bate-Smith test**

In the bate smith test, ethyl acetate fraction of banana skin showed positive results because there was a dark red color occurs (Figure 2.). These changes color indicate that the skin of banana contains flavonoids.



**Figure 2** Bate-Smith Test Result

3.3.3.2 Tannin Test

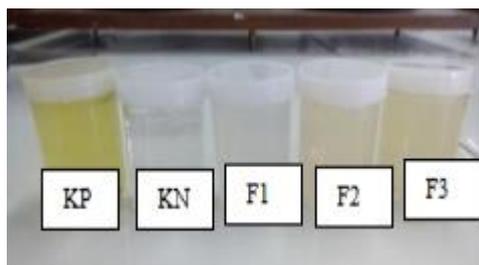
Ethyl acetate fraction showed positive results in this test because there was a blackish green color change when the addition of FeCl<sub>3</sub>. Mechanism change of color is tannins react with Fe<sup>3+</sup> ions to form complex compounds [11].



**Figure 3** Tannin Test Result

*3.3.4 Formulation of serum gel with ethyl acetate of fraction banana skin.*

Formulation of Serum Gel Formulation with Ethyl Acetate Fraction of Banana skins. The result of formula serum gel with ethyl acetate fraction shows Figure 4.



**Figure 4** Formula KP, KN, F1, F2 and F3.

*3.3.5 Physical Test of Serum Gel Ethyl Acetate Fraction of Banana Skin.*

3.3.5.1 Organoleptic

The Organoleptic examination was carried out by a descriptive method. Organoleptic results show in table 2.

**Table 2** Organoleptic test serum gel of fraction banana skin result

Formula	Result				
	Color	Odor	Shape	Spreadability (cm)	Homogeneity
F1	Cloudy White	Spesific Banana Odor	Semisolid	6,6±0,1	Homogen
F2	Cream	Spesific Banana Odor	Semisolid	6,6±0,635	Homogen
F3	Light Brown	Spesific Banana Odor	Semisolid	5,9±0,152	Homogen
KN	Clear color	Spesific Carbopol Odor	Semisolid	6,8±0,152	Homogen

Serum gel without ethyl acetate fraction (negative control) had clear color. The variation in the concentration of the ethyl acetate fraction of banana skin in formula did not affect the odor of the serum gel formula. The serum gel formula had a specific banana odor . All serum gels were made had a soft texture, spread easily, semisolid consistency, and do not feel sticky. The values of spreadability indicate that the gel is easily spreadable by small amount of shear [12].

The homogeneity test results showed that the serum gel formula did not feel rough or lumpy when touched, and there were no small powders so it could be said to be physically homogeneous. This indicates that the difference in the concentration of the ethyl acetate fraction of banana skin does not effect the homogeneity of serum gel.

3.3.5.2 pH of Serum Gel

PH measurement results are shown in Table 3 . The ideal pH value of the preparation in accordance with the pH of the skin is 4.5-7 (SNI 16-4399-1996). If the formulation too acidic, it will cause skin irritation. If the preparation too alkaline, it will cause dry and scaly skin [13]. Based on the results of pH measurements, it is known that the serum gel ethyl acetate fraction of banana skins have a pH that matches the requirements of topical preparations.

**Table 3** Results Serum Gel pH Measurement

Formula	pH mean ± SD
F1	4.5 ± 0
F2	5 ± 0
F3	5 ± 0
K N	5 ± 0

**3.3.5.3 Serum Gel Viscosity Measurement**

The aim of measuring the viscosity of serum gel is to determine the viscosity value of a formula. The results of the viscosity measurements average serum gel formula shows in Table 4.

**Table 4** Results Serum Gel Viscosity

Formula	Mean viscosity (cps) ± SD
F1	12230 ± 199.7498
F2	13630 ± 635.9245
F3	15730 ± 425.6759
K N	11340 ± 443.9595

The viscosity value of gel serum according to SNI 16-4399-1996 is in the range 2000-40000 cP. Based on the measurement results, the four serum gel ethyl acetate fraction of banana skin had a viscosity between 11340 to 15730 cps. So it can be concluded that the viscosity gel serum is qualifying into the viscosity range.

The difference in the concentration of the ethyl acetate fraction of banana skins was different (p <0.05). The higher concentration of the fraction added makes the greater the viscosity.

**3.3.6 Stability Testing Of Serum Gel With Cycling Test Method**

Cycling Test method aims to test the stability of serum gel formula and is carried out for 12 days or as many as 6 cycles. The Cycling Test result method can be see in table 5.

Based on the table 5, formulations 1, 2, 3 and KN showed no color change after the 6th cycle. This indicates that the serum serum gel formula stable. Formula remains from the beginning of the cycle to the 6th cycle. However, in positive control, there was a change when the 6th cycle, was initially light yellow to dark yellow and has low value viscosity (table VI). This is because in the positive control contain vitamin C can cause oxidized process in the presence of oxygen. It is make viscosity decrease [14].

The pH of the serum gel was examined at the beginning and end of the cycle. According to SNI No.16-4399-1996, the range of pH values before and after the cycling test was still within safe limits for topical

preparations. Based on the table above, the formulations 1, 2, 3 and negative controls did not change the pH (table 6). This results shows that the serum gel formula was stable.

**Table 5** Results Of Organoleptic Stability Testing Of Serum Gel Using The Cycling Test Method

Formula	Observation			
	Color		Shape	
	The start of the cycle	Cycle-6	The start of the cycle	Cycle-6
F1	White cloudy	White cloudy	Semi solid	Semi solid
F2	Cream	Cream	Semi solid	Semi solid
F3	Light brown	Light brown	Semi solid	Semi solid
KN	Clear	Clear	Semisolid	Semisolid
KP	Light yellow	Dark yellow	Semisolid	Liquid

**Table 6.** The results of testing the stability of pH and viscosity of serum gel using the Cycling Test method

Formula	Observation			
	pH		Viscosity	
	The start of the cycle	Cycle-6	The start of the cycle	Cycle-6
F1	4.5	4.5	12330	11880
F2	5	5	13530	11220
F3	5	5	15270	13470
KN	6	6	10830	8520
KP	6	7	9870	6060

**3.3.7 Antioxidant activity test of serum gel formula.**

**3.3.7.1 Result of Determination of Maximum Wavelength (λ max) of DPPH**

The measurement results show that the maximum DPPH 0.1 mM wavelength was 517 nm with an absorbance of 0.899.

**3.3.7.2 Antioxidant Activity of the Ethyl Acetate Fraction of Banana Skin.**

The DPPH method measures the ability of a compound to scavenge free radicals. The ability of antioxidants is related to the ability of compound components to donate electrons or hydrogen. Any molecule can donate electrons or hydrogen will react to DPPH. This mechanism will change the color of the solution, from purple to yellow [15].

Antioxidant activity is the ability of a compound or an extract to inhibit oxidation reactions which can be expressed by the presentation of DPPH absorption. The value of IC50 is obtained from the relationship between percent inhibition and concentration [16]. Table VII shows category antioxidant

activity according to IC50 value. Table 7 shows the result of antioxidant activity F1, F2, F3, KN and KP.

**Table 7.** Category Antioxidant Activity

IC50 Value (ppm)	Category Antioxidant Activity
<50	Very strong
50-100	Strong
100-150	Moderate
150-200	Weak

**Table 8.** The result of antioxidant Activity

Formulations	Average IC 50 (ppm) ± SD
F1	87.947 ± 0.962
F2	84.297 ± 1.165
F3	71.257 ± 1.139
K N	205,699 ± 2,455
KP	55.595 ± 1,727

From these results, it can be concluded that a serum gel of F1, F2, and F3 had strong antioxidant activity. Gel serum with various concentrations had higher antioxidant activity when compared with the negative control group. One-way ANOVA statistical test shows that the IC50 value of the three formulas had a significant difference ( $p < 0.05$ ) compared with the negative control. This means that the serum gel formula has antioxidant activity. The three formulas when compared with positive control show significant differences ( $p < 0.05$ ). The serum gel of the ethyl acetate fraction of banana skins at all concentrations had less antioxidant activity than the positive control group. This was estimated because of the inadequate release of active substances from the base when reacting with DPPH. Other factors such as environmental factors like light which can cause an oxidation process which results in a decrease in the antioxidant activity of the preparation [17].

**4. CONCLUSION**

The ethyl acetate fraction of banana skin can be formulated in serum gel and have a good value in physical properties, spreadability, pH, and viscosity. The serum gel is stable to the cycling test and F3 is a formula that has great antioxidant value.

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