Antioxidant Activity of Nanoemulsion Gel of Rambutan Fruit Peel Extracts (*Nephelium lappaceum* L.) Using Dpph and FTC Method

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Abstracts

Rambutan fruit peel extracts had been reported to have quite high antioxidant activity. There are several compounds classes of phenolic and flavonoid contained in Rambutan fruit peel extracts such as ellagic acid, corillagine and geraniine that are responsible for its antioxidant activity. Antioxidant compounds are commonly used in cosmetic preparations. This study was conducted to explain the antioxidant activity of rambutan fruit peel extracts in nanoemulsion gel formulation which was tested with DPPH (1.1-diphenyl-2-picrylhydrazyl) and FTC (Ferric Thiosyanate) method. A method of DPPH was done by making 5 series of concentration and read as absorbance at λ max by using spectrophotometer UV-Vis and for the method of FTC, it was read as absorbance at λ 500 nm. The result of absorbance could be used to determine the value of IC_{50} (DPPH method) and inhibition percent (FTC method). The result of the study using DPPH method obtained the IC_{50} values of nanoemulsion gel of Rambutan fruit peel extracts in the amount of 9.32 ± 0.06 mg/mL, which was higher than X® Sunscreen Gel and vitamin E with IC_{50} value of 40.41 ± 0.98 mg/mL and 10.41 ± 0.05 mg/mL, respectively. The results with the FTC method obtained the percent value of inhibition of 51.09 ± 0.99%, which was lower than X® Sunscreen Gel and vitamin E with percent value of inhibition of 60.07% ± 13.23% and 79.07 ± 7.62%, respectively. These results indicated that Rambutan fruit peel extracts had quite high antioxidant activity that after they were formulated into a nanoemulsion gel.

Keywords: rambutan fruit peel extracts, antioxidant activity, nanoemulsion gel, DPPH and FTC method

1. Introduction

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit that can be found in almost all regions in Indonesia. Usually, people only consume the fruit, while the peels are discarded as waste. The study used the waste of rambutan fruit peel to be extracted. Based on studies that had been done, rambutan fruit peel extract has quite high antioxidant activity (Muhtadi et al., 2014). Antioxidants are substances that have the ability to inhibit, delay, prevent or slow down the free radical reactions with donor mechanism of one or more electrons to free radicals (Winarsi, 2007). Antioxidants alone can be obtained by natural or derived from synthetic. One of the natural antioxidant is rambutan fruit peel extracts.

Some phenolic compounds and flavonoids are contained in rambutan fruit peel extracts (*Nephelium lappaceum* L.) such as ellagic acid, corillagin and geraniin. They are compounds which are responsible for antioxidant activity (Thitilertdecha et al., 2010). Based on previous research, rambutan fruit peel extracts also contained compounds known also ethyl gallate compound which is capable of providing higher antioxidant effect than vitamin E. It is established by obtaining DPPH IC_{50} value of rambutan fruit peel extracts of 7.74 ± 0.76 µg/mL and 8.48 ± 0.77 µg/mL of vitamin E (Muhtadi et al., 2014).

Antioxidants are generally used in cosmetic preparations to prevent premature aging. Antioxidants can be well formulated in creams, gels, and lotions (Winarsi, 2007). Cosmetic preparations currently available and more widely used is the form of creams, lotions and gels. Gel preparations is more preferable than creams or lotions form because it is more transparent and has translucent appearance, non-greasy and contains a lot of water that provides cooling sensation and feels pleasant on the skin. Gel form can be made using nanotechnology system resulted to nanoemulsion gel. Nanoemulsion is emulsion system that has a transparent, translucent characterization. It is an oil water dispersion which stabilized by surfactants or film-layer of surfactant molecules which has 50 - 500 nm droplet sizes (Shakeel et al., 2008). The use nanoemulsion gel systems is related to drug delivery systems which aims to improve the bioavailability of active substances. The smaller particle size in a nanoemulsion gel formulation will be more easily penetrated by the skin, increase the absorption of the active substance, increase the penetration through the skin and increase the permeability of the active substance.

The purpose of this study was to determine the antioxidant activity in the nanoemulsion gel of rambutan fruit peel extracts by using the DPPH and FTC method which measured the value of free radical inhibitory activity (IC_{50}) and inhibition percent compared with the antioxidant activity of gel product in the market, namely X® Sunscreen Gel and compared it to vitamin E as a positive control to know the benefits of the peel. Hence, rambutan can be used as a source of natural antioxidants.

2. Materials and Methods

2.1 Instrument

The instruments used in the research were pyrex glass tools (flask, flask, beakers, pipettes volume, glass beaker, stir bar), an analytical balance with a sensitivity of 0.01 mg (Adventure), an analytical balance (Ohaus) UV-Vis spectrophotometer (UV mini-1240 Shimadzu), thickness of 1 cm cuvette (Hellma Analytics), micropipette (Socorex), filter cloth, aluminum foil, oven, vortek, and filter paper.

2.2 Material

The materials were nanoemulsion gel rambutan fruit peel extracts (obtained from previous research), crystal DPPH (*1.1-diphenyl-2-picrylhydrazil*) pa, pa ethanol, 75% ethanol, oleic acid, phosphate buffer pH 7, distilled water, 30% ammonium thiocyanate, $FeCl_2$, nanoemulsion gel without extract, gel comparator "X® Sunscreen gel" and Vitamin E.

2.3 Extraction

A total of 2.8 kg of dry rambutan peels which had been sorted and crushed, macerated in 10 L ethanol 96% for 24 hours while stirred every 1 hour in the first 6 hours. The maserate was evaporated by using a vacuum rotary evaporator to obtain viscous extracts.

2.4 Making of Nanoemulsion Gel Rambutan's Fruit Peel Extract

Nanoemulsion gel of rambutan fruit peel extracts tested was nanoemulsion gel with rambutan fruit peel extract concentration of 255 mg in 100 grams of nanoemulsion gel. The gel formulations were the results that had been made and had the most excellent stability under the parameters gel (Ramadhan, 2016).

2.5 Antioxidant Activity Test of DPPH Methods

The sample of nanoemulsion gel of approximately 1 gram rambutan fruit peel extracts that was placed above the porcelain dish was weighed and dissolved in 10 mL ethanol p.a (extract concentration 255 ppm). Then the researchers made a series of concentrations (5; 7.5; 10; 12.5 and 15 µg / mL). Several series of concentrations were then pipetted 2 mL respectively into 5 mL flask. In each flask, it added 2 mL of DPPH solution (0.2 mM) then added ethanol p.a up to the mark. Wait for 30 minutes at room temperature (25^0C). The mixture then was measured by using UV-Vis spectrophotometry at λ maximum (Molyneux, 2004). The experiments were repeated for 3 times. The same treatment was also applied on nanoemulsi gel without extracts, gel comparator "X® Sunscreen Gel" and vitamin E.

2.6 Antioxidant Activity Test of FTC Methods

The sample of nanoemulsion gel of approximately 1 gram rambutan fruit peel extracts that was placed on a porcelain dish was weighed and dissolved in 10 mL ethanol p.a in the flask. Vitamin E that was weighed for 10 mg on a watch glass was taken 4 mL then dissolved with ethanol p.a to 10 mL in the flask. The solution was measured by using a measuring pipette, put into a flask and added with 4.1 mL of 2.52% oleic acid, 3.9 mL of distilled water and added phosphate buffer pH 7 to 25 mL in the flask. This mixture was called the stock solution and incubated at 40^0C in the oven. The stock solution was taken 0.1 mL and added 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 0.02 M $FeCl_2$ in a flask and added ethanol 75% v/v to 10 mL and incubated at room temperature for 3 minutes. It was read at a wavelength of 500 nm (Rezaeizadeh et al., 2011). The measurements were done every 24 hours until the absorbance of control (a mixture of 2.52% oleic acid, phosphate buffer pH 7, distilled water and solvent extracts) reached a maximum. The same treatment was also applied on the nanoemulsion gel without extracts, gel comparator "X® Sunscreen Gel", and vitamin E.

2.7 Analysis of Data

The obtained data results of antioxidant activity (IC_{50} and % inhibition) of nanoemulsion gel of rambutan fruit peel extracts, nanoemulsion gel without extracts, gel of "X® Sunscreen" as the comparator and vitamin E as positive control were analyzed statistically. ANOVA test was done to compare the mean of antioxidant activity between groups. It used IBM SPSS version 23 application, which had a 95% confidence level.

3. Results and Discussion

3.1 Antioxidant Activity Testing of DPPH Method

The basic principle of DPPH method is the color changes in DPPH free radicals as the result of their hydrogen atom (H) donation process of the antioxidant compounds (test samples) which will react with DPPH. This will cause a decrease in absorption intensity of the DPPH color that was originally purple to yellow. The more hydrogen atoms (H) are donated, DPPH color will fade into light yellow.

This study resulted in a decrease of DPPH color intensity on the sample of nanoemulsion gel of rambutan fruit peel extracts, gel comparator X® Sunscreen Gel and positive control vitamin E. However, on X® Sunscreen Gel, the intensity color decrease is not so significant. This result showed that the three samples provided antioxidant activity against DPPH due to the process of contributing hydrogen atom (H) donor against free radicals, which caused a decrease in the intensity of the color. This was unlike the case with nanoemulsion gel without extract that did not show a decrease of color intensity. This indicated that these samples did

not have a donor activity of hydrogen atoms (H) against free radicals, which meant that they had various roles as a negative control. There was an absence of the extract or compounds that acted as active substances in the gel that led to none of antioxidant activity.

Based on IC_{50} values that were obtained from the four samples, nanoemulsion gel of rambutan fruit peel extracts obtained IC_{50} value of 9.32 µg/mL. As for the comparison of X® Sunscreen Gel IC_{50} value of 40.41 µg/mL was obtained and vitamin E which acted as a positive control obtained IC_{50} value of 10.41 µg/mL. The lower the IC_{50} values obtained, the greater the antioxidant activity of a sample. Reynertson (2007) classified the IC_{50} value into 4 sections namely IC_{50} of less than 50 µg/mL was listed very active, 50-100 µg/mL was listed active, 100-200 µg/mL was listed quite active and more than 200 µg/mL was listed inactive as an antioxidant. IC_{50} value obtained from nanoemulsion gel of rambutan fruit peel extracts, gel of X® Sunscreen and vitamin E was less than 50 µg/mL. Therefore, it could be concluded that they had a very active antioxidant activity. These results were supported by the AAI values obtained from three samples. AAI value or antioxidant activity index is a value that categorizes antioxidant activity to be weak, medium, strong and very strong based on the value of AAI obtained. AAI value was calculated by using the formula where DPPH concentration (ppm) that was used was divided by the obtained IC_{50} (µg/mL). It was considered as weak or inactive if the value of AAI < 0.5, moderate if 0.5 < AAI < 1.0, strong if 1.0 < AAI < 2.0, and very strong if the value of AAI > 2.0 (Scherer and Godoy, 2009). AAI values obtained for nanoemulsion gel of rambutan fruit peel extracts was 8.58 (very strong), nanoemulsion gel without extract was 0.08 (not active), X® Sunscreen Gel was 1.98 (strong) and vitamin E was 7.68 (very strong).

Table 1. Value IC_{50} and AAI

Sample	IC_{50} (µg/mL)	AAI (*Antioxidant Activity Index*)
Nanoemulsion Gel Rambutan's Fruit Peel Extract	9.32 ± 0.06	8.58
Nanoemulsion Gel Without Extract	968.24 ± 420.74	0.08
X® Sunscreen Gel	40.41 ± 0.98	1.98
Vitamin E	10.41 ± 0.05	7.68

Nanoemulsion gel of rambutan fruit peel extracts have high antioxidant activity because it contains phenolic and flavonoid compounds such as ellagic acid, corillagin and geraniin which are responsible for antioxidant activity (Thitilertdecha et al., 2010). The class of phenolic compounds and flavonoids such as ellagic acid, corillagin and geraniin are known to have a hydroxyl group (OH) attached to an aromatic ring. Hydroxyl group (OH) attached to an aromatic ring will donate a hydrogen atom (H) to free radicals to stabilize it. Vitamin E also has a high antioxidant activity because it has a hydroxyl group (OH) attached to an aromatic ring so it can donate a hydrogen atom (H) to free radicals, even though a hydroxyl group (OH) of vitamin E is as not much as ellagic acid, corilagin and geraniin configuration and total hydroxyl groups are the foundation that greatly affect its activity as an antioxidant mechanism (Mokgope, 2007).

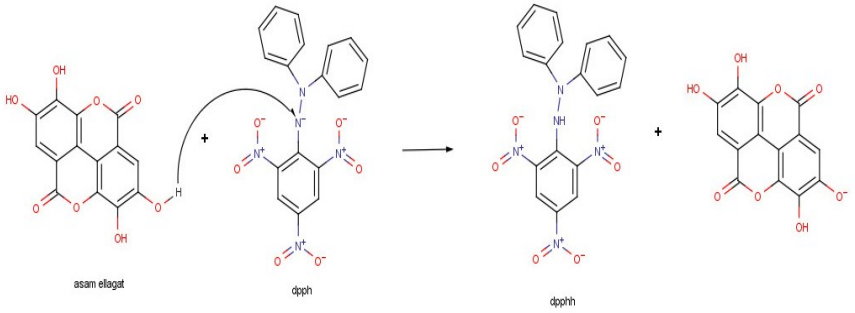

Figure 1. Inhibition of DPPH free radical reactions by ellagic acid into DPPH

In the studies that had been reported by Muhtadi et al. (2014), it showed that rambutan fruit peel extracts had radical inhibitory activity value (IC_{50}) of 7.74 ± 0.76 µg/mL by using DPPH method. In this study, the rambutan fruit peel extracts had been formulated in a gel form nanoemulsion and the obtained value of radical inhibitory activity (IC_{50}) was 9.32 ± 0.06 µg/mL. There was no significant difference between the rambutan fruit peel extracts with the extracts that had been formulated into a nanoemulsion gel. This showed that the gel formulation nanoemulsion could maintain the antioxidant activity of the rambutan fruit peel extracts. In measuring the antioxidant activity of nanoemulsion gel without extract, it was obtained that the IC_{50} value was 968.24 ± 420.74 µg/mL, which indicated the absence of the antioxidant activity. There was significant difference between the gel nanoemulsion rambutan fruit peel extracts by gel without extract nanoemulsion which was seen from the significant value of 0.003 ($p < 0.05$). This result showed the effect of the extract in nanoemulsion gel that caused the antioxidant activity. On the other hand, the nanoemulsion gel of rambutan fruit peel extracts, X® Sunscreen Gel and vitamin E showed no significant differences that were seen from the significant value of both were equally 1.000 ($p > 0.05$). However, it was found that the nanoemulsion gel had lower IC_{50} values than the X® Sunscreen Gel as gel comparator and vitamin E as positive control.

3.2 Antioxidant Activity Test of FTC Methods

FTC method is based on the inhibition of lipid peroxidation reaction. FTC method was done by measuring the amount of peroxide in the process of lipid peroxidation and thiocyanate complexes formed ferry reactions were read at a wavelength (λ) of 500 nm. Unsaturated fats would lose its hydrogen atoms (H) in CH_2 group resulting unpaired carbon atoms (CH) then occured a chain reaction. Therefore, the antioxidant compounds in the sample would break the chain reaction in a way donor the hydrogen atom (H) (Devasagayam et al., 2004). Low absorbance showed high antioxidant activity of free radicals that were formed during stable lipid peroxidation. Radicals formed were relatively stable due to resonance, and it was not easy to partake in a chain reaction (Nijveldt et al., 2001).

The reading of lipid peroxidation process was done until the maximum absorbance values obtained from controls. Radical inhibition profile was tested for 6 days with a range of reading of samples every 24 hours.

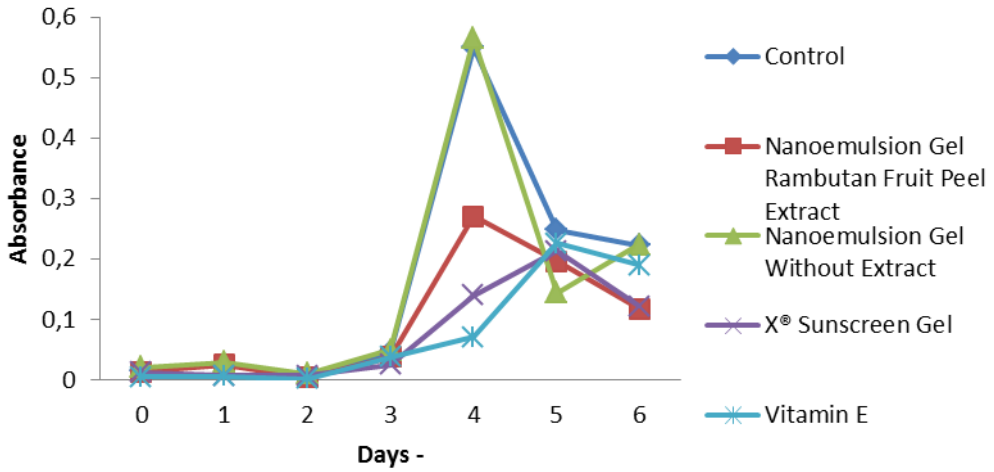

Figure 2. Profile absorbance value; control, nanoemulsion gel rambutan fruit peel extracts, nanoemulsion gel without extracts, X® Sunscreen Gel and Vitamin E

The samples, which were tested, showed antioxidant activity when the absorbance values were obtained under control absorbance. Nanoemulsion gel of rambutan fruit peel extracts, X® Sunscreen Gel and vitamin E were shown to have antioxidant activity due to the absorbance value obtained under control absorbance. There were some similarities between the absorbance value profile nanoemulsion gel of rambutan fruit peel extracts with X® Sunscreen Gel, while for nanoemulsion gel without extract, the obtained absorbance values was greater than the control.

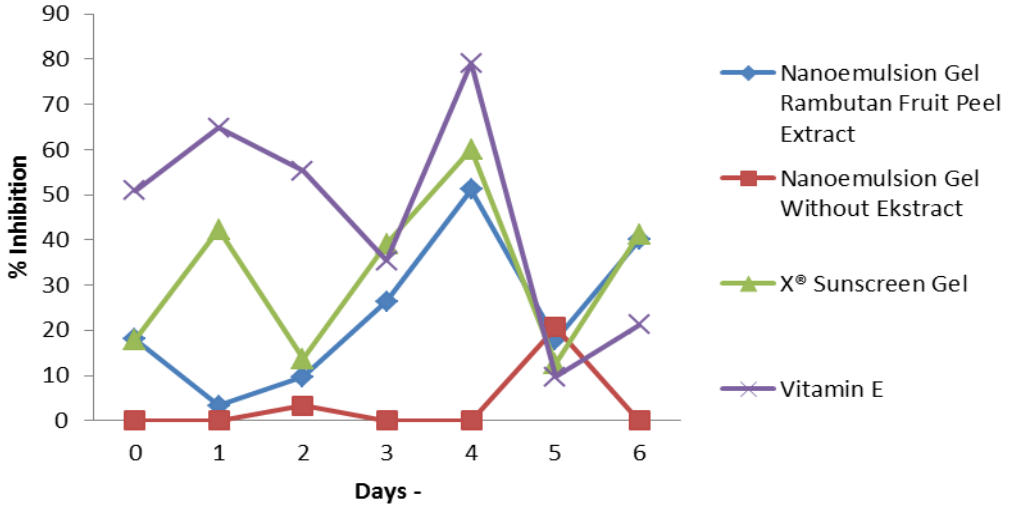

Figure 3. Profile increase in the averagepercent inhibition FTC method; nanoemulsion gel rambutan fruit peel extract, nanoemulsion gel without extract, the gel comparator of X® Sunscreen Gel and positive control vitamin E

The results showed conformity to the reaction mechanism in which control would had the highest absorbance value. This was caused by the absence of an antioxidant agent in control as a radical inhibitor; therefore, it produced more Fe^{3+} (III) and the color of solution after the

addition of ammonium thiocyanate would become redder than the addition of a standard extract or vitamin E on the stock solution.

Table 2. Percent Inhibition of FTC method on the fourth day

Samples	Abs	Abs Control	% Inhibition
Nanoemulsion Gel Rambutan's Fruit Peel Extracts	0.252		51.09 ± 0.99
Nanoemulsion Gel Without Extracts	0.527	0.51	0
X® Sunscreen Gel	0.203		60.07 ± 13.23
Vitamin E	0.106		79.07 ± 7.62

Table 2 showed that the percent inhibition of FTC method on all samples tested on the fourth day at which the absorbance values obtained for maximum control. Vitamin E had the highest value of inhibition percent, which was 79.07 ± 7.62%, followed by X® Sunscreen Gel as gel comparator with the value of 60.07 ± 13.23%, and the nanoemulsion gel of rambutan fruit peel extracts that had the value of percent inhibition of 51.09 ± 0.99%. There was no significant difference between the nanoemulsion gel of rambutan fruit peel extracts with X® Sunscreen Gel seen from the significant value, which was equal to 1.000 ($p > 0.05$).

Based on the results, the antioxidant activity of nanoemulsion gel of rambutan fruit peel extracts was shown by the test results of DPPH and FTC method. When it was compared to the X® Sunscreen gel, it had no significant difference with a significance value equally of 1.000 ($p > 0.05$). This showed that the nanoemulsion gel of rambutan fruit peel extracts had been similar to the X® Sunscreen Gel in terms of antioxidant power, which meant that nanoemulsion gel of rambutan fruit peel extract was feasible to be used as an antioxidant cosmetic preparations. The big difference in the measurement of antioxidant and mechanism ways between the two methods led to differences in the results of antioxidant activity (Zahin et al., 2009). The content of phenolic and flavonoid classes of compounds in the nanoemulsion gel of rambutan fruit peel extracts were responsible for antioxidant activity by using both DPPH and FTC method. Class of phenolic compounds and flavonoids such as ellagic acid, corillagin and geraniin were hydrogen atom donors for free radicals (DPPH) and the free radicals were formed during lipid peroxidation (FTC).

Rambutan fruit peel extracts were known to have high levels of phenolics and flavonoids of 373.19 mg/g GAE and 12.26 mg/g QE, respectively; however, it had low antioxidant activity, which was examined by using the FTC method in the amount of 22.06% (Hidayati et al., 2014). The weakness of FTC method was the formation of complex color that tended to fade if the steps were not done quickly thereby affecting the accuracy of the result despite its high sensitivity. The steps were sufficiently long since the researchers had to change the unsaturated fatty acid (oleic acid) into a radical compound first then it was reacted with the addition of $FeCl_2$ and thiocyanate to form thiocyanate complexes, which would also affect the accuracy of obtained results. In addition, the required high skill and the appropriate time for the distance measurements were narrow (Yamaguchi et al., 1983). The result of antioxidant testing indicated that the nanoemulsion gel of rambutan peel extracts by using the FTC method was higher than the rambutan fruit peel extracts without nanoformulated that was equal to 51.09%. These results indicated that the formulation of nanoemulsion gel could increase the antioxidant activity of rambutan fruit peel even though the cause of the increase was still not known for certain. It was necessary to assay total phenolic and flavonoid in nanoemulsion gel of rambutan peel extracts

quantitatively in order to know the correlation between the levels of phenolic compounds and flavonoids of rambutan fruit peel extracts in nanoemulsion gel to its antioxidant activity.

4. Conclusion

The nanoemulsion gel of rambutan fruit peel extracts had high antioxidant activity with IC_{50} values by DPPH method of 9.32 ± 0.06 mg / mL and the percent value of inhibition obtained from the FTC method amounted to 51.09 ± 0.99%. The nanoemulsion gel of rambutan fruit peel extracts with IC_{50} was higher than X® Sunscreen Gel as comparator and vitamin E as positive control; however, it had lower percent of inhibition activity than the both.

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