Indonesian J. Pharm. Vol. 27 No. 3 : 145 – 151
ISSN-p : 2338-9427
DOI: 10.14499/indonesianjpharm27iss3pp145

Research Article

ANTIOXIDANT ACTIVITY OF DRIED STRAWBERRY JUICES (*Fragaria vesca* L.) EMULGEL PREPARATION USING CANDLENUT OIL AND IT'S DIFFUSION

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Submitted: 11-04-2016
Revised: 12-06-2016
Accepted: 13-08-2016

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Strawberry (*Fragaria vesca* L.) is a fruit that contains nutrients with many benefits and used as an antioxidant because it contains phenolic, ascorbic acid, pelargonidin-3-glucoside and cyanidin-3-glucoside. In addition, strawberries contain flavonoids, which have high antioxidant capacity. Double emulsion can improve the stability of phenolic compounds from dried strawberry juice. Primary emulsion is dispersed in a gel base (emulgel) and it is expected to produce more stable and better preparation with the ability to release the active ingredient topically. The production of double emulsion preparation (A / M / A) using candlenut oil as the oil phase with high level of total flavonoid and total anthocyanins of formula respectively - were 22.86% and 55.915%. The total flavonoid in dried strawberry juice emulgel was able to pass the membrane shed snake skin with higher flux value than the total flavonoid in the form of emulsion type A / M.

Key words: formulation, emulgel, strawberry juice, difussion, shed snake skin

INTRODUCTION

One of the fruits that can be used as an antioxidant is strawberry (Fragaria vesca L.). Strawberries have phenolic content, ascorbic acid (71.2±4.9mg/100g), pelargonidin-3-glucoside (254.1±33.6pg/g), and cyanidin-3-glucoside (9.8±1.8ug/g) (Crecente-Campo *et al.,* 2012). High content of ascorbic acid, phenolic, flavonoid and anthocyanin has important role for the antioxidant activity (Shin *et al.,* 2007; Mosquera *et al.,* 2012).

Strawberries also contain coenzyme Q10 of 1.4ug/g of wet weight which has antioxidant activity. It can improve skin health, skin repair and improve skin regeneration (Borekova *et al.,* 2008). The ethanol extract of red strawberry fruit in Sweet Charlie varieties and Camarosa shows the EC50 value (mg/mL) and each value was 58.05±2.48 and 39.01± 4.92, while for the acetic acid extract was respectively 0.2%, 9.71±4.96 and 29.86±5.80 (Mandave *et al.,* 2014). Strawberries can be used as an antioxidant because it has dye anthocyanins, namely pelargonidin3-O-glucoside and cyanidin3-O-glucoside which dissolved in water (Wang *et al.,* 2014). In addition, strawberries contain flavonoid that has high antioxidant capacity (Jin *et al.,* 2011). Strawberries also have an effect as an anti-melanogenesis, so that they can prevent pigmentation of the skin by reducing the tyrosinase activity and melanin content in the melanocytes. As the result, it can brighten the skin (Zhu *et al.,* 2015).

The use of a combination of hydrophilic and lipophilic surfactants can form stable double emulsion (Akhtar *et al.,* 2010). The primary emulsion which formed is dispersed in Carbopol. Carbopol is a gelling agent that can be used in stabilizing the emulsions by lowering the surface tension and increases the viscosity of the water phase (Khullar *et al.,* 2012). Candlenut oil is used as the oil phase because it contains high oleic acid that has function as enhancers. Oleic acid is able to increase the permeability of the skin by influencing the composition of the stratum corneum which is hydrophobic so that it can increase the penetration (Kogan and Garti, 2006).

It is necessary to do research on the use of strawberries in the form emulgel in order to obtain more benefits, to be more efficient and more practical. In addition, by making the emulgel preparation, it is expected to become more stable with the ability to release the active ingredient topically better. From this research, the release of the active ingredient was tested

through the transport test through membranes shed snake skin. This method was chosen because it has stratum corneum content which is similar to human, and it is also expected to describe the release of emulgel strawberries preparation when it is used topically (Itoh *et al.,* 1990).

The purpose of this study is to make preparation to improve the stability of the antioxidant content of dried strawberry juice. Preparation is expected to improve the ability of the release of active ingredients topically by transport through membranes with shed snake skin test.

MATERIALS AND METHODS

Research Materials

Strawberries (*Fragaria vesca* L.) are from Banyuroto village, hazelnut oil, tween 80 (Bratachem), span 80 (Bratachem), propylene glycol (Bratachem), Croduret 50ss (Croda), Carbopol (Bratachem), PEG 400 (Bratachem), HCl (Sigma-Aldrich), ethanol (Sigma-Aldrich), raw quercetin, aquabidest (Bratachem), CH3COONa, KCl, AlCl3, no.1 Whatmann paper, filter paper, PBS buffer solution pH 7.4; citrate buffer pH 5.4; membrane shed snake skin.

The determination of the antioxidant activity of dried strawberry juice

Strawberry making done freeze dry using freeze dryer. Lost of drying content measured using moisture balance equipment at 105°C. Dried strawberry fruit juice were carefully weighed in 500mg of and 125.7mg of raw quercetin dissolved with 96% ethanol p.a in ad 50mL flask. This solution was pipetted and diluted. Next, each solution is added with 0.4mM DPPH and 96% ethanol p.a until it reached mark boundaries, it was shaked until it became homogeneous mixture for 30min. The absorbance of this solution was measured at 517 nm wavelength. Then,the absorbance of reading negative control and a positive control was determined by using quercetin.

Determine the percentage of flavonoid and anthocyanin of strawberry fruit juice

Level of flavonoid: dried strawberry juice was pondered and diluted with 96% ethanol in a flask and filtering. Then, read absorbance of the solution at maximum wavelength.

The determination of total anthocyanins level: dried strawberry juice was pondered and reconstituted using 0.1% HCl -etanol 96% (8.5 to 1.5mL). Each 1mL solution was taken and put in a 50mL flask separately. Then each solution was diluted using buffer solution pH 1 and pH 4.5. the absorbance of each solution is measured at a wavelength of 700nm and at the maximum point. The result of absorbance is then calculated using the formula (Lee *et al,.* 2005 and Tonutare *et al.,* 2014):

$$A=(A\lambda_{maks}-A\lambda_{700nm})pH1-(A\lambda_{maks}-\lambda_{700nm})pH4.5$$

The total anthocyanin is calculated using the following formula:

$$\text{TotalAnthocyanins}\left(\frac{mg}{L}\right)=\left(\frac{A \times MW \times DF \times 100}{\varepsilon \times I}\right)$$

Where ε is 36000L /mol, MW is the molucular weight, I is the cuvette optical pathlengeht (1cm) and DF is the dilution factor.

The formulation formula of emulsion preparation type A / M and emulgel (double emulsion) of dried strawberry juice

Emulsion preparation was made using three emulsifier components, such as span 80, croduret 50ss, and propylene glycol. Dried strawberry juice was dissolved with HCl 0.1%-PEG 400 (3:1) with pH 3 and it was used as water phase. The water phase mixed with propylene glycol. Then, the oil phase was made by mixing the candlenut oil with span 80 and croduret 50ss. The water phase was poured little by little in the oil phase and it was stirred by stirrer 1500rpm for 20min (emulsion type A/M). Next, carbopol was dispersed in the water and stirrer with 750rpm. TEA and tween 80 were added into gel. Emulsion type A/M was added into gel phase and stirred with stirrer 500rpm until it became emulgel (double emulsion).

Determine the percentage of flavonoid and anthocyanins emulgel formula

The determination of flavonoid: 1g of emulgel and 96% of ethanol was dissolved (stock). Then, from the stock solution, 1.0mL was taken and 96% of ethanol was added ad 50mL p.a. The absorbance readings at maximum wavelength.

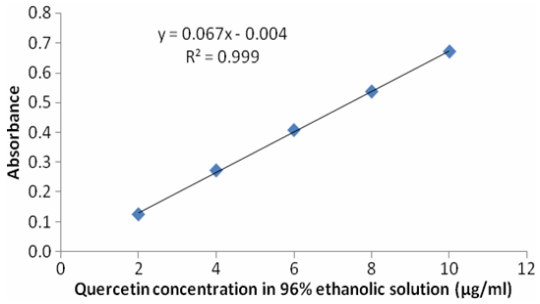

Figure 1. The calibration curve of standard solution of quercetin in 96% ethanol at 2-10ug/mL concentration

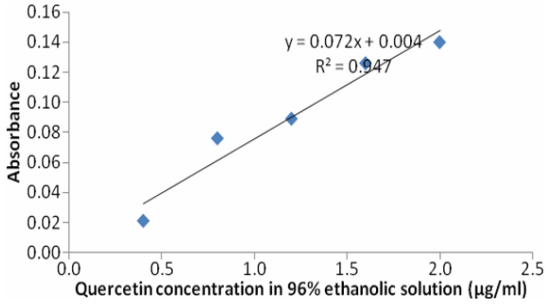

Figure 2. The calibration curve standard solution of quercetin in 96% ethanol at 0.4-2 ug/mL concentration

Table I. The percentage of inhibition of radical DPPH from various series concentration of dried strawberry juice

Dried Strawberries Juice Concentration (mg / mL)	% Inhibition of radical DPPH
80	24,682
160	27,070
240	48,567
480	63,694
640	77,866

The lower level of flavonoid was calculated by using the linear regression equation as result of standard curve of quercetin.

Determination of total anthocyanins level: it was carefully pondered 1.0g of emulgel and dissolved in 10mL using HCl 0.1%: etanol 96% (8.5: 1.5). Each solution was taken 1.0mL and added to the pH 1.0 solution using 0.025M KCl solution and pH 4.5 using 0.4M acetate buffer, and then the absorbance was measured at a wavelength of 496nm and 700nm. The total anthocyanin level was calculated using the equation.

The membrane transport test through shed snake skin

The membrane transport test in this study was done by using Franz diffusion cell, which has 1.4cm2 area of diffusion capacity, 1.0g donor compartment and the receptor compartment volume 26.0mL. The receptor compartment filled with saline phosphate buffer with pH 7.4 and stirred using a magnetic stirrer 100rpm, then used a shed snake skin with buffer cellophane coated membrane as membrane. The transport test is done by taking a sample of 2.0mL of the receptor compartment using a syringe and replaced with a solution of phosphate buffer at pH 7.4 for 8h. The sample of solution was taken then its absorbance was measured with UV-VIS spectrophotometry at maximum wavelength at 377nm (Ibrahim *et al.,* 2007; Khullar *et al.,* 2012; and Kuramoto *et al.,* 1996).

RESULT AND DISCUSSION

Determination of antioxidant activity of dried strawberry juice

The Determination of flavonoid was done quantitatively using UV-Vis spectro-photometry (Figure 1 and 2). The making processs of standard curve was done by dissolving the quercetin in ethanol 96% at a wavelength of 377nm. Quercetin in alcohol has a maximum wave-length at 258 and 375nm (Budavari, 1996).

The equation on the compound quercetin standard calibration curve was used as a benchmark in the quantitative analysis on the measurement of total flavonoid content in dried strawberry juice. In the test the antioxidant activity of strawberry fruit, the regression equation $y = 0.096\ x + 17.54$ ($R^2 = 0.953$) with IC_{50} values of dried strawberry juice at 0.338mg /mL was obtained.

Table II. Physical changes of emulsion stability in storage condition

Period of Storage (days)	Storage Temperature (°C)	Changes Happened
0	25	No changes in color and form emulsion
	4	No change in color and form emulsion
7	25	No changes and emulsion form
	45	Pale pink color, no changes in the form of an emulsion
	4	Pale pink color, not deformed emulsion
14	25	Pale pink color, not deformed emulsion
	45	Pale pink color, cracking occurs

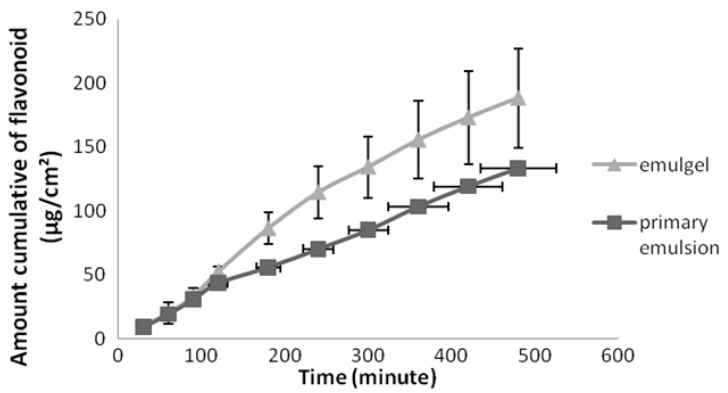

Figure 3. The relationship curve of amount cumulative of flavonoid (ug/cm2) through shed snake skin membrane of emulgel and the primary emulsion of dried strawberry juice.

The regression equation y=27.67 x-31.07 (R^2=0.995) was obtained with the raw quercetin test. The value IC_{50} quercetin from the result of research was 2.93ug/mL. From the research, by comparing IC_{50} between dried strawberry juice toward the antioxidant activity of quercetin, It was obtained that dried strawberry juice 1/115 times weaker than the antioxidant activity of quercetin (Table I). This is because quercetin is a pure compound so that it has greater activity.

Determination of flavonoid and anthocyanin of strawberry fruit juice

The relationship between the content of quercetin in ethanol 96% pa and its absorbance was stated as the equation y=0.067 x-0.004 with a correlation coefficient (R^2=0.9997). The test result of flavonoid dried strawberry juice was 22.86% w/w, which means 228.6mg of flavonoid in 1g of dried strawberry juice.

The content of anthocyanin in strawberries with various conditions of pH and absorbance was measured at a wavelength of 496nm and 700nm in the solution for pH 1.0 using 0.025 M KCl buffer solution and acetate buffer pH 4.5, it was used as a blank 0.4m in calculating the total of anthocyanin content. The results showed that the anthocyanin content was 55.915% or 559.15mg / 1g of powder of dried strawberry juice.

Preparation process of emulsion formula type A / M and emulgel (double emulsion) of strawberry fruit juice

The making process of dried strawberry juice formula emulgel was done in several stages with the determination of the primary emulsion base and materials which were used as the first stage. Further primary emulsion formed was dispersed into the gel base.

Preparation of primary emulsion changes when it was stored for 14 days in organoleptis, such as change in color, pH and viscosity of emulgel preparation, this change occured due to the degradation of anthocyanins and quercetin.

Table III. Values of Q cumulative flux and permeability coefficient of total flavonoids through shed snake skin membrane

Parameter	Emulgel	Primer Emulsion
Q cumulative	188.298 μg	133.214μg
The flux	0.0032μg/cm^2/sec	0.0029μg/cm^2/sec
The permeability coefficient	8.402x10^{-5}cm/sec	7.58x10^{-5}cm/sec
The diffusion coefficient	2.184x10^{-5}cm^2/sec	1.971x10^{-6}cm^2/sec

Organoleptic assessment of emulgel preparation

Organoleptic evaluation was done toward the emulgel to see the characteristics of emulgel. The evaluation of characteristics of the primary emulsion and emulgel included the color preparation which produce, odor, and homogeneity. The colour of primary emulsion preparation changed during 14 days of storage, the previous color, it was the original pink which similar to homogeneous solution, it was visually interesting and there was no precipitate formed and then its color changed into pale pink. From its smell and stickiness parameter when it smeared on the skin surface, it showed that the typical smell of hazelnut oil with 80 high concentration of span, and it gave the impression of stickiness on the skin surface. From the homogeneity parameter indicates the primary homogeneous emulsion preparation (Table II).

The fade of pink color in the preparation of primary emulsion caused by reduction of anthocyanin level. Anthocyanin is red pigment which is in the strawberries. The anthocyanin level in improper storage condition reduces and due to the increase in temperature (Wang *et al.,* 2014)

The measurement of pH

The results of pH measurement primary emulsion of dried strawberry juice on a storage temperature of 4°C, 25°C and 45°C for 28 days increased the pH. Primary emulsion which was stored either at 4°C, 25°C and 45°C for 28 days experienced a significant rise in pH statisticaly using single sample t-test ($p < 0.05$). The increase in pH during the storage also occurred in the emulgel preparation along with storage duration in the conditions of 25°C. The pH emulgel (double emulsion) of dried strawberry juice increased in the storage condition for 28 days. It may because of the influence of the temperature and the emulsifier content which influenced by pH.

Determination formula of flavonoid and anthocyanin

The measurement of flavonoid in dried strawberry juice emulgel on first day, it was 13.89% and its content decreased in the storage condition for 30 days at 4°C, 25°C and 45°C respectively 2.97%; 5.35% and 2.08%. The decrease in flavonoid due to the influence of temperature. While the results of measurement of anthocyanin content in emulgel on the first day was 7.36%, and in the storage condition, It contained anthocyanin 1.84%. There was a decrease in anthocyanin content during the storage as a result of the increase in the pH of the preparation.

Test of transport membrane through shed snake skin

In the experiment of diffusion through the membrane,it can be seen that only small amount of flavonoid which can be transported through the shed snake skin membrane when it was in the form of primary emulsion and emulgel. The experimental points of trials on emulgel diffusion through the membrane produced linear regression equation $Y=0.193x+30.28$ ($R^2=0.994$) and the primary emulsion produced a linear regression equation $Y=0.174x+3.704$ ($R^2=0.996$). The linear regression equation can be determined Q cumulative, flux, permeability coefficient and diffusion coefficient of flavonoid (Figure 3).

Drug release was related to the stability of the preparation, in case if the coalescence occurs in the internal phase or the broken of oil droplet will affect the release of drug molecules. In the form of double emulsion preparation, the diffusion ability to pass a greasy thin membrane may occur if an oily layer is not semipermeable, and there is an osmotic

gradient factor related to the flow of water molecules (Lieberman, 1998).

The speed of diffusion release on the A/M/A system can be divided into diffusion and rupture of emulsion or membrane rupture, this is influenced by the polarity and molecular weight of the drug and the type or concentration of surfactant.

Diffusion emulsion A/M/A passed three processes, namely the release of drug molecules and the first droplet, the partition between the oil phase and water phase, the movement of the drug into membranes. Diffusion ability of the double emulsion related to the lag time needed to complex partition molecules. The drug release is influenced by the concentration of emulsifier that is lipophilic whereby if the concentration of lipophilic is big, the globules of oil will expand and the drug release is delayed, surfactant lipophilic will diffuse on the surface so that it will give a synergistic effect to increase the strength of the membrane (Dong *et al.,* 2015 and Vasiljevic *et al.,* 2006).

Gel diffusion capability is affected by the size of the pores in the gel, where based on the literature range between 30-40nm so that only the effective diffusion which may happen. The higher the concentration of the gel, the lower the diffusion capability (Quino *et al.,* 2016). The presence of Tween 80 in the preparation emulgel has function to improve the molecules diffusion. The concentration of non-ionic surfactant can increase the permeation of skin because it has the dispersion ability into intracellular lipid phase on the stratum corneum so that it can increase the fluidity and reduce the diffusion barriers (Ibrahim *et al.,* 2007).

According to Ainurofiq (2006), the drug release of emulsion type A/M are more difficult, it may because it is blocked by oily layer so that the barrier layer can not be penetrated by the polar flavonoid, namely hazelnut oil which is non-polar. In releasing the drug by erosion mechanism, that is the depletion of oily layer or broken emulsion. While the double emulsion A/ M/A has a more complicated mechanism that is through coalescence, diffusion mechanism and permeation. Coalescence causes damage to the thin layer between the liquid phase granules in the granular surface of the outer phase or it is common with the term of erosion, while the diffusion mechanism and permeation of chemical substances penetrates the oil phase. The release of flavonoid in the form of double emulsion from the process of breaking up of the oily layer into outer layer, then it penetrates the membrane.

Emulsion type A/M shows the maximum distance when it is trasported and influenced by the size and shape of the droplet, this causes delay diffusion in a long period than when it is in the outer aqueous phase. Diffusion of water which passes through the oily layer is lower when it is compared with oil diffusion in the aqueous layer due to the saturation of the water on the oily layer, while the water diffusion in water-saturated oily phase is influenced by temperature and water solubility (Vermeir *et al.,* 2016).

CONCLUSION

The dried strawberry juice (*Fragaria vesca* L.) has antioxidant activity with IC_{50} value (0.338mg/mL). The production of emulgel dried strawberry juice has not been able to improve the stability of the antioxidant compound content of dried strawberry juice. Emulgel penetration through the shed snake skin membrane is better than the primary emulsion preparation form of type A/M.

ACKNOWLEDGEMENT

The research team would like to thank to the Graduate Grant of Pharmacy Faculty which has provided funds to our research.

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