

## Potency of Super Red Dragon Fruit Flesh Extract (*Hylocereus costaricensis*) in Herbal Lipstick as Colorant, Antioxidant and Antibacterial

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### Abstract

Lipstick is one kind of cosmetics which is used as lips colorant to increase self-confidence. Nowadays, lipsticks from natural source is popular to reduce the negative impact of chemical compound or synthetics colorant in lipstick intensively for health. Super red dragon fruit is one kind of natural ingredients which can be used as colorant for lipstick. Moreover, this fruit is rich of antioxidant and antibacterial component that is good for skin health. In this research, extract of super red dragon fruit flesh was added in the lipstick during preparation with variation of concentration 0, 10, 20, 30 and 40%. Antioxidant analysis resulted the IC<sub>50</sub> value in 81.55% indicating the strong antioxidant properties. Furthermore, the concentration of dragon fruit extract 40% was able to inhibit the growth of *Staphylococcus aureus* bacteria.

**Keywords:** dragon fruit, lipstick, colorant, antioxidant and antibacterial.

### Introduction

Nowadays, cosmetics is intensively used by both men and women as to treat skin, improve the appearance and increase the self-confidence in the community. Lipstick is one kind of cosmetics products which is used to care for and beautify lips. This product is used intensively to keep the colour in lips. Unconsciously, this behaviour increases the possibility of its ingredients to be consumed and accumulated. The accumulation of chemical compounds in the body can inflict the serious health problems, such as allergy, irritation, poisoning, tissue damaging, cancer and death. The synthetics chemical compounds are generally used as colorant, preservatives and flavouring in the ingredients of lipstick. According to this problem, the production of lipstick using safe ingredients from natural products is being developed to maintain our health and prevent the health problem.

Natural ingredients for cosmetics are generally obtained from the plants and

fruits that contain the coloured compounds. One of the natural ingredients for the natural colorants is super red dragon fruit (*Hylocereus costaricensis*). This dragon fruit variety is known to have many benefits for health, such as the high antioxidant content and antibacterial properties. According to Abijeet (2012), super red dragon fruit could be used as the additive in food, medicine and cosmetics. However, the use of this fruit for lipstick is limited. Moreover, the effect of this fruit as antibacterial and antioxidant is not much known. Through this study, the potency of super red dragon flesh will be explored and developed in specific for antioxidant and antibacterial. Its extract will also be developed as a material for a herbal lipstick.

### Material and Methods

#### Samples

The materials in this study consists of 1 kg of super red dragon fruit (*Hylocereus costaricensis*) aged 5 weeks, red colour and fresh. Its rind has been separated from its flesh which give 600 g of its flesh. The fruit flesh is the main component in this study.

#### Extraction of Super Red Fruit Dragon Flesh

Extraction is done in this study by squeezing the fruit flesh, then added 100 mL aquadest and filtered to obtain the extract.

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The half extract was evaporated using rotary evaporator to obtain the crude extract, whilst the half was used to the production of lipstick. The yield was calculated using Equation 1 (Eq.1).

$$\% \text{Yield} = \frac{\text{The weight of obtained extract}}{\text{The initial weight of simplisia}} \times 100\% \text{ (Eq. 1)}$$

### **Phytochemical Screening**

The phytochemical screening was done to detect the flavonoid, saponin, tannin and alkaloid compounds in the sample.

#### *Flavonoid Assay*

Zero point three (0.3) g of fruit flesh extract was placed in the test tube, then added 3 mL n-hexane and shaken roughly until producing no colour in the liquid. Next, 20 mL ethanol 80% was added to the liquid and divided into 3 parts. B1 solution is a blank. B2 solution was added 0.5 mL concentrated HCl then was heated on a water bath. B3 solution was added 0.5 mL concentrated HCl and 4 pieces of magnesium. The changes of colour were observed. The formation of orange solution shows the content of flavonoid (Sangi, Runtuwene and Simbala, 2008).

#### *Saponin Assay*

Zero point three (0.3) g of fruit flesh extract was placed in the test tube, then added 5 mL aquadest and shaken roughly for 30 s. Positive result is shown by the formation of stable bubbles on the surface of the solution for 30 s.

#### *Tannin Assay*

Zero point three (0.3) g of fruit flesh extract was placed in the test tube, then added 10 mL hot aquadest and homogenized. The solution was left at the room temperature, then added 3-4 drops of NaCl 10% and homogenized. The solution was divided into 3 parts. C1 solution is a blank. C2 solution was added 1-2 drops of gelation solution 1% and 5 mL NaCl 10%. The changes of colour were observed. Tannin compound is indicated by the formation of white sediment. If no colour changes were found in C2 solution, 3 drops of  $\text{FeCl}_3$  was added then observed. The formation

of green to orange colour indicates polyphenol content. C3 solution was added 3 drops of  $\text{FeCl}_3$ , the formation of blackish green shows tannin content.

#### *Alkaloid Assay*

Zero point three (0.3) g fruit flesh extract was placed in test tube, then added 5 mL HCl 2N. The solution was heated up for 2-3 min while stirring, then was cooled down and added 0.3 g NaCl and 5 mL HCl 2N. Solution was divided into 3 parts. Solution A1 is a blank, Solution A2 was reacted with 3 drops of Mayer reactant, solution A3 was reacted to Wagner reactant. The colour changes, the formation of precipitant and hazing solution indicates alkaloid compound.

#### *GC-MS Analysis*

GC-MS analysis was done in LIPI Bogor. 1 g crude extract of dragon fruit was added 1 mL dimethyl sulfoxide (DMSO) the homogenized and placed in the ependorf. This sample then was analysed using GC-MS instrument.

#### *Antioxidant Activity Assays of Super Red Dragon Flesh Extract*

This assay was done for liquid extract of dragon fruit using DPPH method (1,1-phenyl-2-picrylhydrazyl). 19.71 mg of DPPH solid was weighted and placed into the volumetric flash. Then 50 mL methanol was added and kept into the dark bottle. Blank solution was incubated in room temperature 27°C for 30 min and wrapped with aluminium foil. For comparing, 100 ppm ascorbic acid was used, maximum absorbance was measured at wavelength 510-520 nm. Sample was calculated at 10, 20, 30, 40 and 50 ppm at 517 nm. Then, the graphic was plotted between % inhibition versus concentration (ppm) which would produce regression equation.  $\text{IC}_{50}$  was calculated in  $\mu\text{g}/\text{mL}$  with y value equal to 50.

#### *Lipstick Preparation*

Eight (8) g of solid lipstick per batch was made using active compound of super red dragon fruit in the variation of crude extract concentration (0, 10, 20, 30 and 40%). The formulation of material for lipstick was shown in Table 1.

Table 1. Formulation of Natural Lipstick

Material	Concentration (%)				
	F0	F1	F2	F3	F4
Crude extract of super red dragon fruit	0	10	20	30	40
Olive oil	33.3	30	26.67	23.33	20
Beeswax	33.3	30	26.67	23.33	20
Peanut Butter	33.3	30	26.67	23.33	20

### Organoleptic Assays

The stability of each lipstick formula was done for 3 weeks with the evaluation every week. Lipstick was kept at 27°C and 37°C. Evaluation were done for the physical appearance (formation of crystal and sweat), smell and texture.

### Peroxide Number Assays of Lipstick

Peroxide number is an indicator to determine the level of oil damage or fat as a result of oxidation reaction in the storage condition. In this assay, peroxide number was done for determine the antioxidant concentration in the lipstick during storage.

### Standardization of $\text{Na}_2\text{S}_2\text{O}_3$ solution.

10 mL  $\text{K}_2\text{Cr}_2\text{O}_7$  was added to Erlenmeyer, followed by 2.5 mL  $\text{H}_2\text{SO}_4$  solution and 5 mL KI 20%. This solution was titrated with  $\text{Na}_2\text{S}_2\text{O}_3$  using amylum as indicator. Titration was performed until dark blue colour disappearing. The concentration of  $\text{Na}_2\text{S}_2\text{O}_3$  was calculated.

### Determination of Peroxide Number.

1 g lipstick was put into the Erlenmeyer flask 50 mL, followed by the addition of 6 mL acetic acid glacial and 0.1 mL potassium iodide saturated. Erlenmeyer was closed and wrapped to prevent from the sun radiation, and leaved it for 1 min while homogenized. 6 mL aquadest was added, the solution was titrated with  $\text{Na}_2\text{S}_2\text{O}_3$  using amylum as indicator until the dark blue colour disappear. Peroxide number of lipstick was determined using equation 2.

$$\frac{\text{Volume of } \text{Na}_2\text{S}_2\text{O}_3 \text{ from titration} \times \text{Normalitas } \text{Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{sample (g)}} = \text{meq/kg} \quad (\text{Eq.2})$$

### Antibacterial Assay

Antibacterial assay was done to know the antibacterial activity of this compound to *Staphylococcus aureus* bacteria. Bacteria was cultured in to Nutrient agar medium (NA) and nutrient broth (NB) and the number of bacteria was calculated for 100 uL medium each petri dish with pour plate method. Plate was group in 4-5 zone to facilitate the treatment. Then, the small holes were made to put the sample. After the sample was put, each petri dish was incubated in 37°C and observed for 24 hours in aerobic condition for *S. aureus*. The ability of antibacterial was observed from the inhibition area which was formed near the holes. The diameter of inhibition area was measured. This assay was done for the formulation of lipstick which was stored for 1 and 3 weeks at either 27°C or 37°C. This assay was also performed for extract of super red dragon fruit and the commercial lipstick.

### Result

#### Extract of Super Red Dragon Fruit.



Extraction produced 450 g liquid extract with red colour (Fig. 1) from 600 gram of super red dragon fruit flesh which gives yield 75%.

Figure 1. Extract of super red dragon fruit (*Hylocereus costaricensis*).

### Phytochemical Screening

Flavonoid, tannin and alkaloid compound was observed in flesh extract of super red dragon fruit, whilst saponin compound was undetectable. The result showed that this extract did not contain saponin. The result of phytochemical assay was shown in Table 2.

### GC-MS Analysis of Super Red Dragon Fruit Extract

GC-MS analysis detected 19 compounds from crude extract of super red dragon fruit (Table 3).

Crude extract of super red dragon fruit contained some compounds which

Table 2. Phytochemical assay result of super red dragon fruit extract

Compound	Parameter	Colour	Result
Flavonoid	Orange red	Orange	+
Saponin	Stable bubble	No bubble	-
Tannin	Yellowish green	Yellowish	+
Alkaloid			
Dragendorff	Red precipitant	Red precipitant	+
Mayer	White precipitant	White precipitant	+
Wagner	Haze	Haze	+

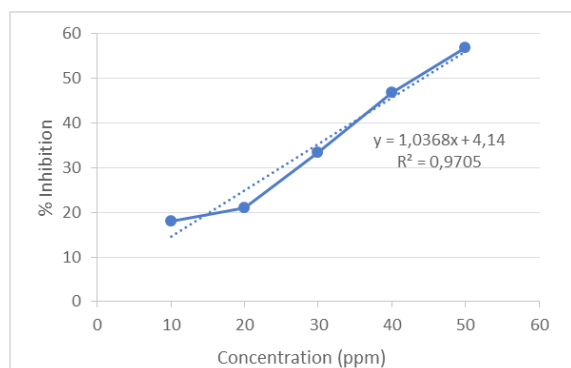


Figure 3. Antioxidant Activity of Super Red Dragon Fruit Extract.

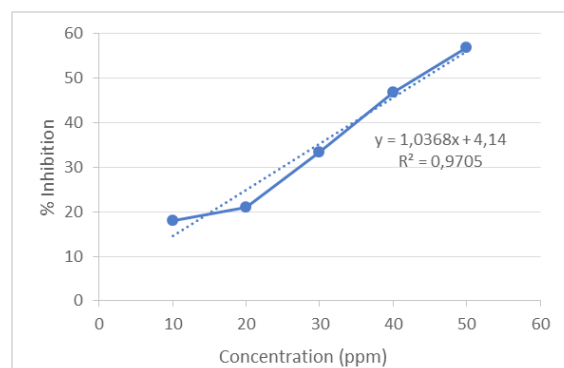


Figure 4. Antioxidant Activity of Ascorbic acid.

have many activities such as antioxidant, preservatives, additive, antimicrobial, antibacterial, insecticide, fungicide and nematicide.

#### ***Antioxidant Assay of Super Red Dragon Fruit Extract***

Antioxidant assay of extract was done using DPPH method. From the assay,  $IC_{50}$  value of this extract was 81.55  $\mu\text{g}/\text{mL}$ . The correlation of % inhibition vs concentration of extract was shown in Fig.3 for dragon fruit extract and Fig. 4 for ascorbic acid as standard.

Ascorbic acid has been known to have antioxidant activity. In this study, we used ascorbic acid as standard which has  $IC_{50}$  value 52.23  $\mu\text{g}/\text{mL}$ . In the comparison of dragon fruit extract with ascorbic acid,  $IC_{50}$  value of the dragon fruit extract is 1.56 times higher than ascorbic acid.

#### ***Organoleptic Assay of Dragon Fruit Lipstick***

Organoleptic assay was performed to obtain the information about the product's stability in texture, physical characteristic, smell and colour. The dragon fruit lipstick

was made from several natural sources such as beeswax, olive oil and peanut butter which was mixed with dragon fruit extract in different concentration. The physical appearance of these lipstick was shown in Fig. 5.

For the physical characteristics, we observed the formation of crystal (C) or no crystal (NC) and sweating (Sw) OR no sweating (NSw). Then, for texture, the product was soft (Sf) or rough (R). In the case of smell, we smell that the product is fragrant (F) or no fragrant (NF). For the colour, we observed if the product is white (W), yellow (Y), pink (P), light purple (LP) or dark purple (DP). The result of organoleptic assay was shown in Table 4.

Most formulation of lipsticks were stable when were kept at 27°C for 3 weeks, except F1 and F2 containing 10 and 20% of crude extract respectively. After 1 weeks, formation of crystal occurred for F1 and F2 that changed their texture becoming rough. The changes of colour happened in all formulation during storage in different time. The colour of F0 and F3 changes at 3<sup>rd</sup> week, whereas F1 is stable. For F2 and F4,

Table 3. Active compounds of super red dragon fruit extract from GC-MS analysis.

No	Compound	Concentration (%)	Activity	Reference
1	Glyceraldehyde	4.2	Intermediate compound in the metabolism of carbohydrate	Najmiatul, 2011
1	2-ethyl-1,3 dioxolane-4-methanol	0.08	Antioxidant	Wrasiati, 2011
2	hexanoic acid, octyl ester	0.45	Antioxidant	Wrasiati, 2011
3	Propanoic acid	0.35	Antioxidant, buffer agent, preservatives and additive	Smith and Hongshum, 2003
4	7-Tetradecene, (E)-	0.13	Antioxidant	Balasundram, Sundram and Samman, 2006
5	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	4.1	Antioxidant	Elezabeth and Arumugam, 2014
6	3-Deoxy-d-mannoic lactone	9.65	Antibacterial	Ghosh, Banerjee and Sil, 2015
7	2-Propenoic acid, oxiranylmethyl ester	0.32	Antimicrobial	Azam, Waris and Nahar, 2005
8	Ethane, 1-chloro-2-isocyanato	0.07	Antimicrobial	Zhao <i>et al.</i> , 2010
9	2-butenic acid, 4,4-dimethoxy-, methyl ester	0.13	Antimicrobial	Wijaya <i>et al.</i> , 2008
10	2-t-butyl-5-propyl-[1,3] dioxolan-4-one	0.29	Antimicrobial	Devi <i>et al.</i> , 2014
11	Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-	0.29	Antimicrobial, preservative	Devi <i>et al.</i> , 2014
12	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.1	Preservatives	Figueiredo <i>et al.</i> , 2008
	Formic acid, 2-propenyl ester	0.78	Preservatives	Yatagai, 2002
13	Nonane, 4,5-dimethyl-	0.14	Preservatives	Yatagai, 2002
14	2-Propanamine, N-methyl-N-nitroso-	3.11	Preservatives	Uma <i>et al.</i> , 2009
15	2(3H)-Furanone, dihydro-3-methylene-	0.03	Preservatives	Uma <i>et al.</i> , 2009
16	[5-Hydroxymethyl)-1,3-dioxolan-4-yl] methanol	1.76	Antibiotic	Aydin, 2005
17	1,2-Benzenediol, 4-methyl-	0.42	Antiseptic	Aydin, 2005
18	(S)-5-Hydroxymethyl-2[5H]-furanone	0.19	Insecticide, fungicide and nematocide	Timothy <i>et al.</i> , 2012
19	Heptanoic acid, 6-oxo-	1.52	Insecticide	Timothy <i>et al.</i> , 2012

% is obtained from the area of sample in the chromatogram which represents the concentration of compound in the sample.

the changes of colour occurred faster than others, which was at 2<sup>nd</sup> week in the storage.

Storage at 37°C was more risk than at 27°C. It resulted the changes of physical and colour faster than at 27°C. The colour of F0 and F2 started to change at 2<sup>nd</sup> week, whereas F1 at 3<sup>rd</sup> week, F3 and F4 at 1<sup>st</sup> week. Formulation of lipstick was faster to changes either physically, texture and colour when it was kept at 37°C compared to 27°C.

#### Peroxide Number Assay

The dragon fruit lipstick was composed of the fatty compounds such as olive oil, beeswax and peanut butter which could undergo oxidation process. This process

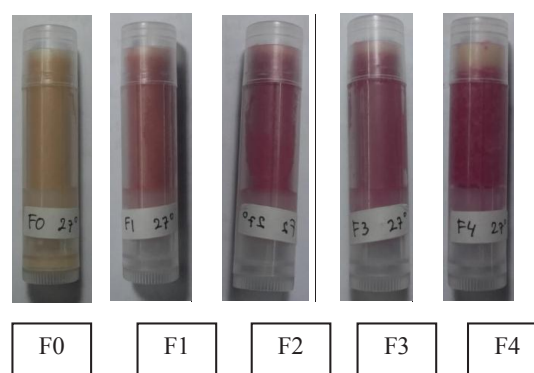


Figure 5. The natural lipstick from the dragon fruit extract. F0: lipstick base, F1: containing 10% of extract; F2: 20% of extract, F3: 30% of extract, F4: 40% of extract.

Table 4. Evaluation of Organoleptic Assay of Super Red Dragon Fruit.

Temperature (°C)	Formulation	Parameter	Week-			
			0	1	2	3
27	F0	Physical	NC/NSw	NC/NSw	NC/NSw	NC/NSw
		Texture	Sf	Sf	Sf	Sf
		Smell	F	F	F	F
		Colour	Y	Y	Y	W
	F1	Physical	NC/NSw	NC/NSw	C/NSw	C/NSw
		Texture	Sf	Sf	R	R
		Smell	F	F	F	F
		Colour	P/LP	P/LP	P/LP	P/LP
	F2	Physical	NC/NSw	NC/NSw	C/NSw	C/NSw
		Texture	Sf	Sf	R	R
		Smell	F	F	F	F
		Colour	LP	LP	P/LP	Y
F3	Physical	NC/NSw	NC/NSw	NC/NSw	NC/NSw	
	Texture	Sf	Sf	Sf	Sf	
	Smell	F	F	F	F	
	Colour	DP	DP	DP	LP	
F4	Physical	NC/NSw	NC/NSw	NC/NSw	NC/NSw	
	Texture	Sf	Sf	Sf	Sf	
	Smell	F	F	F	F	
	Colour	DP	DP	LP	LP	
37	F0	Physical	NC/NSw	NC/NSw	NC/NSw	NC/NSw
		Texture	Sf	Sf	Sf	Sf
		Smell	F	F	F	F
		Colour	Y	Y	W	W
	F1	Physical	NC/NSw	NC/NSw	NC/NSw	NC/NSw
		Texture	Sf	Sf	Sf	Sf
		Smell	F	F	F	F
		Colour	P/LP	P/LP	P/LP	W
	F2	Physical	NC/NSw	NC/NSw	C/NSw	C/NSw
		Texture	Sf	Sf	R	R
		Smell	F	F	F	F
		Colour	LP	LP	Y	W
F3	Physical	NC/NSw	NC/NSw	C/NSw	C/NSw	
	Texture	Sf	Sf	R	R	
	Smell	F	F	F	F	
	Colour	DP	LP	Y	W	
F4	Physical	NC/NSw	NC/Sw	C/Sw	C/Sw	
	Texture	Sf	Sf	R	R	
	Smell	F	F	F	F	
	Colour	DP	LP	Y	W	

Abbreviation C: crystal, NC: no crystal, Sw: sweating, NSw : no sweating, Sf: soft, R: rough, F: fragrant, NF: no fragrant, W: white, Y: yellow, P: pink, LP: light purple, DP: dark purple.

Table 5. Antibacterial Activity of Super Red Dragon Fruit against *Staphylococcus aureus*

Temperature (°C)	Storage period (week)	Inhibition Zone (mm)								
		+	-	E	F0	F1	F2	F3	F4	K
27	1	30	0	14	0	0	0	0	25	0
	3	28	0	14	0	0	0	0	20	0
37	1	28	0	14	0	0	0	0	20	0
	3	25	0	14	0	0	0	0	0	0

(+: Ciprofloxacin, -:Lipstick Base, E:extract, K:Commercial Lipstick)

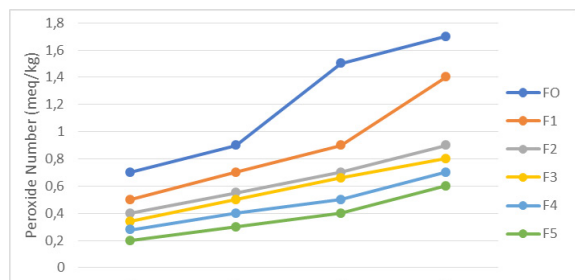


Figure 5. The peroxide number assay of dragon fruit lipstick for storage at 27°C.

could generate bad smell for the product during the storage.

Peroxide number is a number of fatty acid which could undergo oxidation process or hydrolysis producing oxygen and peroxide compound. Through this assay, we were also able to know the antioxidant activity of super red dragon fruit which reduces the bad smell and taste. The result of peroxide number assay was shown at Fig. 5 for storage at 27°C and Fig. 6 for 37°C.

The peroxide number increased in all formulations of lipstick during the storage from 0 until 3 weeks. F0 showed significant increase of peroxide number. The highest peroxide number was observed in F0 with 1.5 meq/kg for 27°C and 1.7 meq/kg for 37°C after observed for 3 weeks. The different result was observed for F1-F5 at 27°C which its peroxide number increased slightly.

In the assay of all formulations of lipstick which were kept at 27°C or 37°C for 3 weeks, the peroxide number reduce along with the increase of extract concentration added into the formulation of lipstick. F1-F5 contained the extract of dragon fruit in different concentration, F5 contain the highest concentration of extract. This result showed that the higher concentration of dragon fruit extract added in the formulation of lipstick, the lower peroxide number of the product which was related with the antioxidant activity of the extract.

The temperature of storage also gives an effect to the peroxide number of lipstick. The result showed that the peroxide number of all formulations at 37°C is slightly higher than 27°C. It presents that the increase of peroxide number is a result of the increase of temperature.

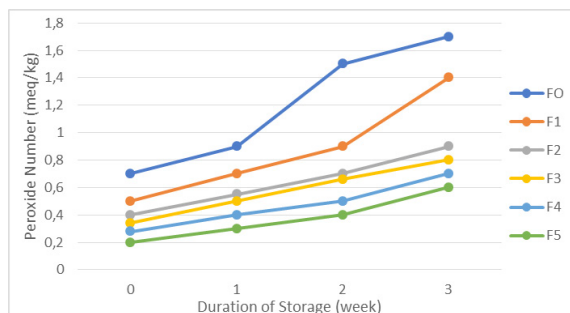


Figure 6. The peroxide number assay of dragon fruit lipstick for storage at 37°C.

### Antibacterial Assay

Dragon fruit has been known as a source of antibacterial. Based on Melliawati (2009), antibacterial in super red dragon fruit was able to overcome the infection of *Staphylococcus aureus*. *S. aureus* is a gram-positive bacterium which grows optimally at 37°C and a normal flora which could be found in skin, respiration system, gastrointestinal and lips.

Ciprofloxacin is an antibiotic used as positive control. Based on the assay, positive control in 1<sup>st</sup> or 3<sup>rd</sup> weeks of storage period for 27°C or 37°C shows the high response in the inhibition of the growth of *S.aureus*. The extract of dragon fruit also represents the inhibition of *S.aureus* growth in both 1<sup>st</sup> and 3<sup>rd</sup> week of storage period. Then, for the formulation of lipstick, F4 shows the positive result for the inhibition of *S.aureus*, whereas another formulation gives same result as negative control even though it contains extract of dragon fruit. In this study, we observed that the inhibition activity of F4 is higher than the extract itself.

### Discussion

#### Secondary Metabolites in Super Red Dragon Fruit

Extract of super red dragon fruit flesh contains many secondary metabolites that have a lot of benefits. Secondary metabolite is an organic compounds that does not have direct function for the growth and development of plant but is needed for the protection against biotic or abiotic stresses and survivals (Pagare *et al.*, 2015). Through phytochemical screening, several secondary metabolites such as flavonoid,

tannin and alkaloid have been detected in this extract.

Flavonoids are the largest group of naturally phenolics compounds isolated from a wide range of vascular plant which act in plant as visual attractors, photoreceptors, stress protectants and for light screening (Pietta, 2000). It also has some benefits for human health such as antioxidants, antimicrobials, antivirals, antiallergenic and anti-inflammatory (Pietta, 2000). Super dragon red fruit has red colour in its flesh and rind. According Harivaindaran (2008), the colour pigment of super red dragon fruit consists of anthocyanin, betalain, betacyanin and betaxanthin. Anthocyanin is a flavonoid group with colour pigment from red to blue. It has some biological activities such as primary antioxidants, chelator and scavenger for superoxide anion.

Moreover, tannin is also detected in the dragon fruit extract. Tannin is a phenolics compounds which have complex structure, difficult to be separated and easy to form crystal. Some studies observed the biological activity of tannin as antioxidant, antimicrobial, antitumor and anti-HIV (Yoshizawa *et al.*, 1987, Ho *et al.*, 1999, Ho *et al.*, 2001, Lü *et al.*, 2004, and Gu *et al.*, 2008).

Other secondary metabolites in dragon fruit is alkaloids. Alkaloids is a compound that contain nitrogen atom in its structure. Alkaloids functionates as antimicrobials, antimalarial, cytotoxic and anti-HIV agents (Ang *et al.*, 2000, Iwasa *et al.*, 2001, and Samoylenko *et al.*, 2009).

Through GC-MS analysis, some secondary metabolites in dragon fruit flesh extract have been detected. Some compounds have antioxidant, antibacterial and antimicrobial as shown in Table 3.

#### ***Antioxidant Activity of Super Red Dragon Fruit***

Antioxidant level of a compound is represented by  $IC_{50}$  value.  $IC_{50}$  represents the concentration of compounds that can prevent and reduce the oxidation reaction in amount of 50%. In this study, DPPH (1,1-diphenyl-2-picrylhydrazyl) method was used to measure antioxidant activity. DPPH shows absorption at wavelength 517 nm.

Some studies has observed the antioxidant activity of dragon fruit. Fidrianny *et al.* (2017), studied the antioxidant activity of super red dragon fruit which obtained  $IC_{50}$  value of ethyl acetate flesh extract of super red dragon fruit in 2.69  $\mu\text{g}/\text{mL}$ . The lowest  $IC_{50}$  indicates the highest antioxidant activity. It could be categorized as a very strong antioxidant.

In this study, the extract of super red dragon fruit flesh has  $IC_{50}$  value 81.55  $\mu\text{g}/\text{mL}$ . This value is higher than vitamin C with  $IC_{50}$  value 52.23  $\mu\text{g}/\text{mL}$ . Based on this result, the antioxidant activity of extract of super red dragon is still lower than vitamin C. However, it can still be classified as strong antioxidant activity.

Super red dragon fruit has antioxidant activity because this fruit contains flavonoids compound which can bind to reactive molecule or free radical species to prevent oxidative reaction.

#### ***Evaluation of Super Red Dragon Fruit Lipstick***

Colour pigment from plant or natural sources has been used in many kinds of cosmetics, included the lipstick. According to Aher *et al.* (2012), herbal lipstick from *Bixa orellana* seeds had minimal or no side effect which mostly arise in synthetic lipstick. In this study, we developed a lipstick formulation using the colour pigment from super red dragon fruit with different concentration. Then, the stability of lipstick was evaluated in 2 different temperatures, 27°C and 37°C.

Our result showed that the storage at 27°C for this lipstick is better than 37°C. The high temperature causes the evaporation faster than low temperature in lipstick formulation which promote the changes in texture, physical appearance and colour. In the case of formulation, lipstick with high concentration of dragon fruit extract, F3 (30%) and F4 (40%), are more stable than others when was kept at 27°C. No changes of physical appearance, texture and smell for F3 and F4 during evaluation 3 weeks, except the colour. F3 was the most stable because the changes of colour occurred lately at 3<sup>rd</sup> week. In accordance with Purbaningtias



*et al.* (2017), anthocyanins pigments (red, purple and blue) are sensitive to the changes of temperature, light exposure and pH. The degradation of anthocyanins was influenced by the condition and storage period of the sample.

#### ***Peroxide Number and Antibacterial Activity of Herbal Lipstick***

Lipstick contains oil and fatty acid which can undergo hydrolysis or oxidation causing rancid smell. The oxidation process is affected by oxygen availability, light exposure, high temperature, the kind of fatty acid, and storage condition (Ketaren, 1986). In this study, peroxide number assay was performed to measure the number of fatty acid which can undergo oxidation reaction. This assay used iodometric titration for 3 weeks storage.

The result of this assay showed high peroxide number in the lipstick formulation without the dragon fruit extract (base formulation) which was kept either at 27°C or 37°C. It was contrast to the base formulation, lipstick formulation containing dragon fruit extract had low peroxide number. It represented that this flesh extract possesses antioxidant compounds encountering the oxidative reaction. It was correlated with the concentration of extract in the lipstick. The higher concentration of extracts which is added to the formulation, the lower peroxide number that was detected. In GC-MS analysis, several compounds having antioxidant activity have been detected (Table 3). According to Winarsi (2007), antioxidant compounds are able to stop oxidant reaction and the formation of free radical molecules.

Apart from being antioxidant, super red dragon fruit has also antibacterial activity. According to Khalili *et al.* (2012), red pitaya or dragon fruit flesh and peel has have potential against pathogenic food microorganisms, gram positive or negative bacteria. For *S. aureus*, the extract of red pitaya showed high activity of inhibition with the inhibition zones  $19.00 \pm 0.43$  mm.

The extract of super red dragon fruit flesh resulted the inhibition zones 14 mm against *S. aureus*, whilst the ciprofloxacin

around 28 mm and F4 formulation which was stored for 3 weeks at 27°C around 20 mm. It showed that F4 formulation stored at 27°C has an antibacterial activity against *S. aureus*. F1, F2 and F3 did not show any inhibition caused by lower concentration of extract. In the contrast with dragon fruit lipstick F4, the commercial lipstick did not show any antibacterial activity. Inhibition zone which is higher or around 20 mm indicates the inhibition response of *S. aureus* growth (Brooks, 2007). This result corresponded to (Khalili *et al.*, 2012), that red dragon fruit flesh has antibacterial activity, in particular *S. aureus*. Then, based on the study, storage temperature affected the activity of compounds in the inhibition of *S. aureus* growth. No antibacterial activity of F4 lipstick formulation after stored at 37°C for 3 weeks, whilst at lower temperature, 27°C, the antibacterial activity was still high in F4 formulation. Although F1, F2, and F3 have also contained the extract of dragon fruit, no antibacterial activity has been observed for these formulations at both storage temperature. It represents that the concentration of extract less than 40% in the sample of lipstick could not inhibit the growth of *S. aureus* effectively. The effect of storage temperature needs to be studied in detail.

#### **Conclusion**

The extract of super red dragon fruit flesh can be used as colorant in herbal lipstick with storage period for 3 weeks at 27°C and 2 weeks at 37°C. The antioxidant activity of this extract was 81.55 µg/mL. This activity was also indicated by low peroxide number of super red dragon fruit lipstick at different concentration of extract. The best formulation for this herbal lipstick was F4 formulation. This formulation showed the high stability when was stored at 27°C for 3 weeks. Moreover, F4 formulation had an antibacterial activity against *S. aureus* when was kept at 27°C.

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