

## Formulation of Blush Preparations by Using Natural Coloring from Red Beetroot Extract (*Beta vulgaris* L.)

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**Abstract:** Beetroot (*Beta vulgaris* L.) has compounds that can be used for body health, beauty skincare, food additives, and much more. This research was aimed to prepare the dry extract of beetroot and formulate it into a loose powder, compact powder, and cream. The preparation was started by adding 2, 4, or 6% of dry extract, then blending the pulp and drying the resultant residue using a freeze dryer. Testing on color homogeneity, polishing, breakage, pH stability, color stability, and the hedonic test was carried out to determine the product quality. The initial result of phytochemical screening showed it might contain flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, and quinones. The color stability test performed at 30 °C showed that the cream was unstable while other forms showed fair stability at 8 °C. All dosage forms were homogeneous and could be applied easily. The breakage test showed no fractures. The pH remained stable for all formulas (between 3–5) after 28 days of storage. The color stability test showed that the significant discoloration only happened to the loose powder and cream. The hedonic test showed that the compact powder with a concentration of 6% was the most preferred formula by users.

**Keywords:** beetroot; *Beta vulgaris* L.; blush on; loose powder; compact powder; cream

### ■ INTRODUCTION

A research on the consumer buying behaviour towards cosmetic products in 2015 in Pune city showed that of 200 consumers of cosmetic products, 60% preferred to buy organic cosmetics and 42.5% used the cosmetic products for the beauty [1]. Similarly, a research regarding the consumers behavior towards the cosmetic products in Delhi [2] stated that the factors that motivate consumers to buy the cosmetic products were influenced by the culture and social life of consumers as well as their psychological condition that might be affected by the advertisement they saw about cosmetic products [2].

Cosmetics are any substances or unit doses intended to be applied on the entire exterior of the human body including teeth and the mucous membranes around the mouth [3]. Previous research showed that 73% consumers

used cosmetics as protection to their skin and others used it as fashion (37%) and to attract people (19%) [4]. Another reason why consumers use cosmetics is to treat disease on their skins [5].

Based on their characteristics, the cosmetic powders can be classified into 2 types, namely loose powder, and compact powder. The particle size of the compact powder is generally greater than that of the loose powder. The dust cloud may be formed during the handling or the use of loose powder so that the safe inhalation should be anticipated. The compact powder is expected to be safer than loose powder, due to the compressed format and the more practical application to the skin [6-7].

Herbal cosmetics are plant-derived-cosmetics which refer to products formulated using several types of cosmetic ingredients that are permitted to form a

basis on which one or more herbal ingredients are used [8]. Previous research showed that some herbal plants were the right choice in treating various disorders on the skin such as inflammation, aging, eczema as well as irritation [9].

Beetroot (*Beta vulgaris* L.) not also can be used as a herbal treatment and a source of antioxidant but it also serves as a natural colorant in food due to the presence of betalain in the extract derived from the root and stem parts of this plant [10-12]. A betalain is a water-soluble flavonoid compound, which has two groups, namely red betacyanin and yellow betaxanthin [13-14]. Previous research [15] successfully used betalain from the beetroot extract to deliver red color in Indian sweet food. However, the instability under heating conditions is one of the shortcomings of betacyanin which is affected by pH, temperature, and oxidation. Elevated temperatures and long heating time can cause decomposition and structural changes in pigment and lead to discoloration [13].

In general, natural colorants have no toxic or allergic properties and show fewer side effects. Their biodegradable properties make them safer to use than the synthetic dyes which sometimes may damage health and environment [16-17]. Natural colorants serve as antimicrobial, UV protection, deodorizing finishing, moth resistant, and food coloration. It has been classified into six classes based on application methods which are mordant dyes, vat dyes, direct dyes, acid dyes, basic dyes, and disperse dyes [18]. Anthocyanins, ones of the important compounds contained in natural colorants, are abundantly available in nature and have many biological activities such as fostering the health of eyes and improving the stability of capillary [19].

This research was aimed to prepare the dry extract of beetroot and to formulate the extract as blushes in the forms of loose powder, compact powder, and cream. The freeze-drying method was done to obtain dry extracts while maintaining the color stability of betacyanin [13]. The beetroot extract was obtained by blending and filtering it to get the filtrate, then drying it using a freeze dryer. The content of betacyanin in the beetroot can be used as a dye in dosage forms. The formulation of blushes in the form of loose powder, compact powder, and cream

was aimed to enhance the potency of the natural color by adding 2, 4, or 6% of dry extract into each formula. This research was aimed to explore the potential of beetroot (*Beta vulgaris* L.) as a natural colorant agent, especially for red color, and the application when it formed as compact powder.

## ■ EXPERIMENTAL SECTION

### Materials

The materials used in this study included: fresh beetroot (*Beta vulgaris* L.) obtained from Pasir Mulya organic Indonesia farm, talcum (90% purity, Pingdu Talc Mine, Shandong), oleum ricini (87.4% purity, Fagron, Rotterdam), acidum ascorbicum (99.8% purity, CSPC Weisheng Pharmaceutical), magnesium stearate (Faci Asia Pacific, Singapore), methylparaben (100.3%), vaseline album, oleum cacao (Bumi Tangerang Mesindotama, Tangerang), adeps lanae (Fagron, Rotterdam), 99.9% propylene glycol, cera alba (Caesar Loretz, Germany), cetyl alcohol (Ecogreen Oleochemicals, Jakarta), iron(III) chloride solution (Merck, Germany), formaldehyde 30% (Merck, Germany), HCl (Merck, Germany), NaOH (Merck, Germany), Mg<sub>2</sub>SO<sub>4</sub> (Merck, Germany), *n*-amyl alcohol (Merck, Germany), acetic acid (Merck, Germany), sulfuric acid (Merck, Germany), chloroform (Merck, Germany), sodium acetate (Merck, Germany), Dragendorff reagent, Mayer reagent and distilled water (Brataco).

### Instrumentation

pH stability was measured at 25 °C and 8 °C using the Delta 320 pH meter (Mettler-Toledo, Schwerzenbach, Switzerland). Color stability was measured at 25 °C and 8 °C using skin color probe (dermalab combo, Denmark).

### Procedure

#### **Preparation of the dosage forms from beetroot extract**

The fresh beetroot (1 kg) was washed using running water to remove the dust and dirt. The clean material was cut into small pieces then blended and filtered to separate it from the residue. The pH of the

filtrate was determined. The pH should be in the range of 4–6 to produce stability [20]. The filtrate with a stable pH was then dried using a freeze dryer to obtain the dry extract. The next process was phytochemical screening to identify the compounds contained in the extract [21-22].

### **Phytochemical screening**

**Identification of tannin.** In a beaker glass, 40 mg of extract was added into 100 mL of boiled water for 15 min and the mixture was left for a while so that it could be filtered using filter paper. The filtrate obtained was divided into two parts, each of 5 mL, and put into a test tube.

On tube 1:

A few drops of 1% iron(III) chloride solution was appended to form a blue-green violet precipitate indicating the presence of tannin compounds.

On tube 2:

The filtrate was added with 15 mL of Stiasny's reagent (formaldehyde 30%:concentrated HCl = 2:1) then heated on a water bath to form a pink precipitate which indicated the presence of condensed tannins. Having filtered, it was saturated with sodium acetate powder and then added a few drops of 1% iron(III) chloride solution to show the presence of hydrolyzed tannins.

**Identification of flavonoid.** As much as 40 mg of extract was added to 100 mL of hot water, the mixture was boiled for 5 min, then filtered to be used as the test solution. The solution (5 mL) was put into a test tube and then added 0.1 mg of powder or magnesium plate and 1 mL of concentrated HCl, and 5 mL of *n*-amyl alcohol. Having vigorously shaken, the mixture was allowed to separate. The formation of red, yellow or orange color in the amyl alcohol solution indicated the presence of flavonoids.

**Identification of saponin.** The extract (40 mg) was diluted with 20 mL of water, put into a test tube, shaken vertically for 10 sec and left for 10 min. The results showed a formation of a stable foam in the test tube. As an indication of the presence of the saponin group, the foam remained stable when 1% of HCl solution was added into the test tube.

**Identification of steroid and triterpenoid.** The extract (20 mg) was macerated with 20 mL ether for 2 h

(in a container with a tight lid), and then filtered. The filtrate (5 mL) was evaporated in the evaporator cup until a residue was obtained. The residue was then added with two drops of anhydrous acetic acid and a drop of concentrated sulfuric acid (Liebermann-Burchard reagent). While the formation of green color indicated the presence of steroid, the formation of red color indicated the presence of triterpenoid.

**Identification of alkaloid.** The extract (40 mg) was added to 5 mL of the concentrated ammonia solution (30%). The mixture was then grinded in a mortar, then added with 20 mL of chloroform and grinded again strongly. The mixture was filtered with filter paper to obtain a filtrate in the form of an organic solution (as solution A). Solution A (10 mL) was extracted with 10 mL of HCl solution 2 M in a ratio of 1:10 by shaking in a test tube and then the upper layer was taken (as solution B). The filter paper was dripped with a few drops of solution A then sprayed and then dripped with Dragendorff reagent. The formation of red-orange color on the filter paper showed the presence of alkaloids. Solution B was divided into two test tubes, each was added with Dragendorff and Mayer reagents. The formation of brick-red sediment on the Dragendorff reagent and white precipitate on the Mayer reagent, showed the presence of alkaloids.

### **Preparation of compact powder**

The comparison of each composition of the blush formula in the form of compact powder can be seen in table 1. The blush was made into 10 g dosage form. The ingredients including dry extract of beetroot CP2; CP4; CP6 (0.2; 0.4; 0.6 g) oleum ricini (0.25 g); ascorbic acid (0.2 g); magnesium stearate (0.5 g); methylparaben (0.1 g) and talcum CP2; CP4; CP6 (8.75; 8.55; 8.35 g) were weighed. The weighted extract was then mixed and grinded with a portion of talcum in a clean mortar until it became homogeneous and dry. The next step was to mix the other ingredients and grinded again until it became homogeneous and soft for about 15–20 min to disperse it completely, and then print it in a container to make it dry. The powder was then placed in a tightly closed container.

**Table 1.** The formulas of beetroot extract into blushes dosage forms

Material	Formula (%)								
	Compact Powder			Loose Powder			Cream		
	CP2%	CP4%	CP6%	LP2%	LP4%	LP6%	C2%	C4%	C6%
Extract (Coloring agent)	2	4	6	2	4	6	2	4	6
Mg Stearate (Adhesive to skin)	5	5	5	5	5	5	-	-	-
Metil Paraben(Antimicrobial)	1	1	1	1	1	1	1	1	1
Ascorbic Acid (Antioxidant)	2	2	2	2	2	2	2	2	2
Oleum ricini (Binding)	2.5	2.5	2.5	1.5	1.5	1.5	20	20	20
Adeps lanae (Emulsifying agent)	-	-	-	-	-	-	5	5	5
Cetyl alcohol (Emulsifying agent)	-	-	-	-	-	-	2	2	2
Oleum cacao (Basis cream)	-	-	-	-	-	-	15	15	15
Cera alba (Basis cream)	-	-	-	-	-	-	10	10	10
Propylene glycol (Humectant)	-	-	-	-	-	-	10	10	10
Vaselin album (Basis cream)	-	-	-	-	-	-	20	20	20
Talcum (Basis powder and compact powder)	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	-	-	-
Aquades (Solvent)	-	-	-	-	-	-	Ad 100	Ad 100	Ad 100

Notes : \*) LP2% = Loose powder with beetroot extract 2%

LP4% = Loose powder with beetroot extract 4%

LP6% = Loose powder with beetroot extract 6%

CP2% = Compact powder with beetroot extract 2%

CP4% = Compact powder with beetroot extract 4%

CP6% = Compact powder with beetroot extract 6%

C2% = Cream with beetroot extract 2%

C4% = Cream with beetroot extract 4%

C6% = Cream with beetroot extract 6%

### Preparation of loose powder

The comparison of each composition in the blush formula in the form of loose powder can be seen in Table 1. The procedure has no difference with the compact powder except at the last stage. The blush was made into 10 g dosage. The ingredients including the dry extract of beetroot LP2; LP4; LP6 (0.2; 0.4; 0.6 g); oleum ricini (0.15); ascorbic acid (0.2 g); magnesium stearate (0.5 g); methyl paraben (0.1 g) and talcum LP2; LP4; LP6 (8.85; 8.65; 8.45 g) were weighed. The weighed extract was then mixed and grinded with a portion of talcum in a clean mortar until it became homogeneous and dry. The next step was to mix the other ingredients and then grinded again until homogeneous and soft for about 15–20 min to disperse completely it, and then sieved using a sieve (100 mesh). The powder was then placed in a tightly closed container.

### Preparation of cream

The comparison of each composition in the blush formula in the form of cream can be seen in Table 1. The blush was made into 10 g dosage. The ingredients including dry extract of beetroot C2%; C4%; C6% (0.2; 0.4; 0.6 g) oleum ricini (2 g); ascorbic acid (0.2 g); methyl paraben (0.1g); vaseline album (2 g); oleum cacao (1.5 g); adeps lanae (0.5 g); propylene glycol (1 g); cera alba (1 g) and cetyl alcohol (0.2 g) were weighed. The results were then processed into two phases.

**Phase A.** Oleum ricini (2 g); oleum cacao (1.5 g); adeps lanae (0.5 g); cera alba (1 g); vaseline album (2 g) and cetyl alcohol (0.2 g) were melted in a water bath and then stirred until they became homogeneous.

**Phase B.** The extract was dissolved with propylene glycol (1 g) and distilled water (1.3; 1.1; 0.9 mL) then stirred until dissolved. Then, phase A and phase B were

inserted into the mortar and grinded until homogeneous and a cream mass was formed. The mass was then added with ascorbic acid (0.2 g); methyl paraben (0.1 g) and dry extract of beetroot C2%; C4%; C6% (0.2; 0.4; 0.6 g) and the mixture was grinded again until homogeneous.

### Evaluation Tests

#### Color homogeneity test

Homogeneity test was done by applying the sample on a piece of glass or other suitable transparent material [23]. The blushes should show a homogeneous arrangement and show no coarse grains.

#### Polishing test

The polishing test was carried out on all dosage forms in each formula. Each formula was applied to the inner arm three times to observe the color [24].

#### Breakage test

This test was only performed for the compact powder by dropping the powder on the wooden surface three times at a height of 0.2–0.25 m [24].

#### pH stability test

The measurements were made on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> days using a pH meter.

#### Color stability test

The dosage forms stored at 8 °C and 30 °C were tested for the color stability on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup> days using the Dermalab Combo by observing the \*a value [25].

#### Hedonic test

The hedonic test was carried out visually on 20 panelists who had knowledge regarding the assessment method [23]. Each panelist was requested to observe the appearance, texture, smell and color when applied to the skin. The panelists were requested to fill out the questionnaire column that had been given and gave a score as displayed in Table 2.

## RESULTS AND DISCUSSION

### Phytochemical Screening

The initial phytochemical screening showed that the beetroot (*Beta vulgaris* L.) extract might contain flavonoids, alkaloids, saponin, tannin, triterpenoids, steroids (Table 3). The phytochemical screening was

carried out to identify flavonoids in the extract of beetroot (*Beta vulgaris*). Betacyanin, which is the red pigment of the beetroot (*Beta vulgaris* L.), is an example of flavonoids. The presence of flavonoids was indicated by the color change from pink to brownish orange due to the addition of reagents of Mg metal and concentrated HCl to the extract. The treatment was aimed to reduce the benzopyrone moiety in the structure of flavonoids to generate red or orange flavylium salts [26]. The saponins tests showed positive results to all beetroot extracts, which was indicated by the formation of stable foam [26]. Saponins are compounds in plants included in the terpenoid group containing the isoprene framework  $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$ . The presence of saponins can be detected based on their ability to form foam due to their soap-like properties [27]. The tannin test was carried out using the solution of 1%  $\text{FeCl}_3$  reagent. The positive result was marked by changes in color to blackish green which indicated the presence of tannins that reacted with  $\text{Fe}^{3+}$  ions to form complex compounds [26-27]. The results showed that all beetroot extract positively contains alkaloids, which was indicated by the formation of the orange-red precipitate [28] when using

**Table 2.** The preference point used in hedonic scale

Score	Explanation
9	Extremely Like
8	Really Like
7	Like
6	Fairly Like
5	Neutral
4	Fairly dislike
3	Dislike
2	Really Dislike
1	Extremely Dislike

**Table 3.** Phytochemical screening results of *Beta vulgaris* L. extract

Secondary Metabolites	Test results
Flavonoids	+
Alkaloids	+
Steroids	+
Tanin	+
Saponin	+
Steroids and Triterpenoids	+

the Dragendorff reagent and the white precipitate when using the Mayer reagent containing mercuric chloride and potassium iodide. The precipitation occurred since the nitrogen atom in the alkaloids can replace the iodide ion to form the coordination bond with metal ions. In addition, the triterpenoid and steroid tests showed positive results by adding acetic acid and sulfuric acid which might bind to a terpenoid/steroid compound to produce a color change reaction [29].

### Color Homogeneity Test

The homogeneity test is important because it can determine whether the resulting blush preparation meets the aesthetic requirements or not. A good color homogeneity is indicated by the distribution of dyes evenly among the news carriers [25].

The color homogeneity test was carried out on the blushes to determine whether the carrier particles or the dye could mix well to create color when applied to the skin. The homogeneity test results (Table 4) showed a homogeneous structure, and no coarse grains were found.

### Polishing Test

The polishing test was performed to figure out how easy the application of blushes to apply on the skin. Based on the polishing test (Fig. 1), the loose and compact powder produced a soft texture when applied, while the cream had a slightly oily texture. The loose and compact powder showed good results with the pink color at a concentration of 4% and 6% after being applied three times, while the concentration of 2% did not produce a pink color since the level of the extract added was very small. All cream formulas produced a good color by showing red when applied to the inner arm for the first time. The water and oil content of the cream formulation produced a sharper and more pigmented color.

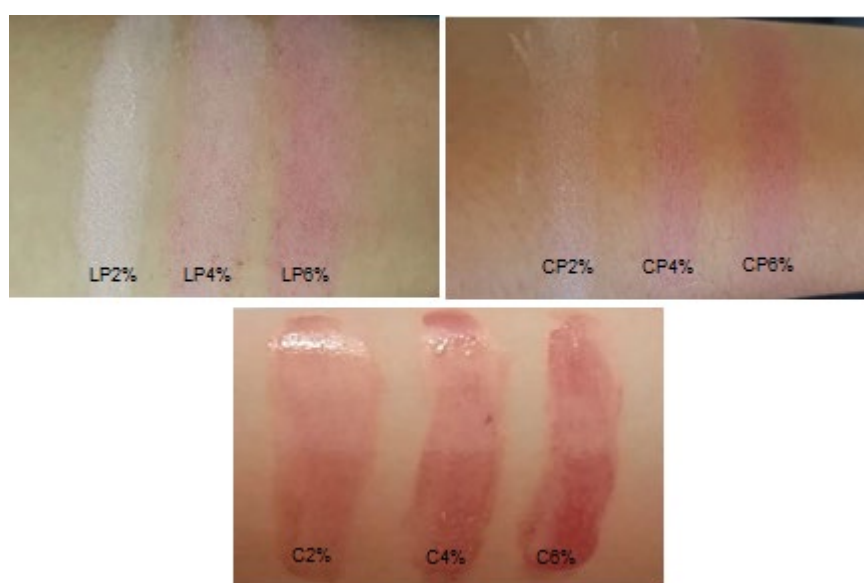
### Breakage Test

In this test, compact powder was dropped to a wooden surface from a height of about 0.2–0.25 m several times to see whether any breakage had occurred on compact powder. The results described in Fig. 2 showed that the compact powder for all formulas remained

**Table 4.** Color homogeneity test results

	Formulas								
	LP2%	LP4%	LP6%	CP2%	CP4%	CP6%	C2%	C4%	C6%
Homogeneity	Good	Good	Good	Good	Good	Good	Good	Good	Good

Notes: LP = Loose powder; CP = Compact powder; C = Cream



**Fig 1.** Polishing test results

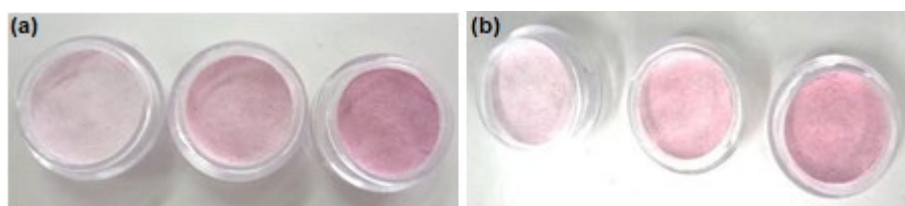


Fig 2. Breakage test results of the blushes compact powder (a) before testing, (b) after testing

compact and unbreakable, indicating that the compactness had passed the test and it was safe in any condition [24].

### pH Stability Test

The pH of human skin is usually acidic, in the range of 4–6, which has historically acted as a defence mechanism against organisms. This steep pH gradient of 2–3 units between the stratum corneum and the epidermis and dermis occur due to the influence of the body internal environment which is close to pH 7–9 (neutral). The physiological role of the skin properties has historically been regarded as a defence mechanism against organisms that attack. Age, skin site, and pigmented skin are some factors that influence the pH of the skin [29].

The pH test was performed at 8 and 30 °C to observe the safety on the skin and also the stability of the dosage so that the difference in the temperature will indicate whether there has been a change of pH during the preparation. The temperatures of 8 and 30 °C are the proper temperatures to observe whether the dosages remain stable in this research. The proper pH of skin plays an important role in maintaining the skin because it

creates a skin barrier and skin resistance to the external physical and chemical agents [30]. After the testing, the loose and the compact powder had average pH of  $\pm 5$ , while the cream had average pH of  $\pm 4$  (Fig. 3 and 4). Therefore, it can be concluded that the loose and compact powder dosage forms met the skin pH requirements and are safe to use on the skin [31]. The pH value of the cream was around  $\pm 4$ , which is lower than the requirements, due to the content of the acidic ingredients of ascorbic acid, oleum, cetyl alcohol, and propylene glycol. It should be noted that the low pH may irritate the skin.

### Color Stability Test

Stable cosmetic preparation is preparation in which the properties and characteristics are the same as at the first time of manufacture during storage and use [33]. The color stability can be represented by the \*a value, which is the value that shows the red color. The higher the \*a value, the redder the test sample will be. The color stability test at 30 °C (Fig. 5) showed that the cream was the most unstable. On the 7<sup>th</sup> day of storage, the cream already showed a color change from dark red

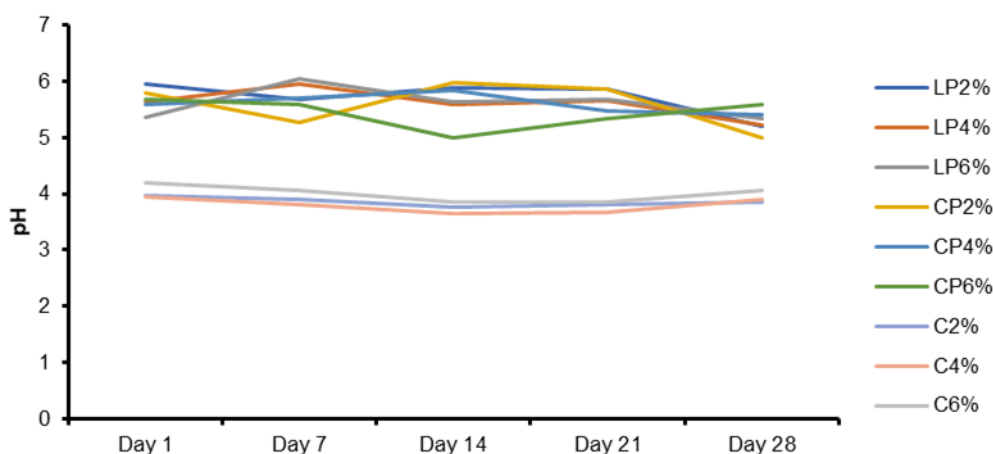


Fig 3. The pH value of the blushes at 8 °C

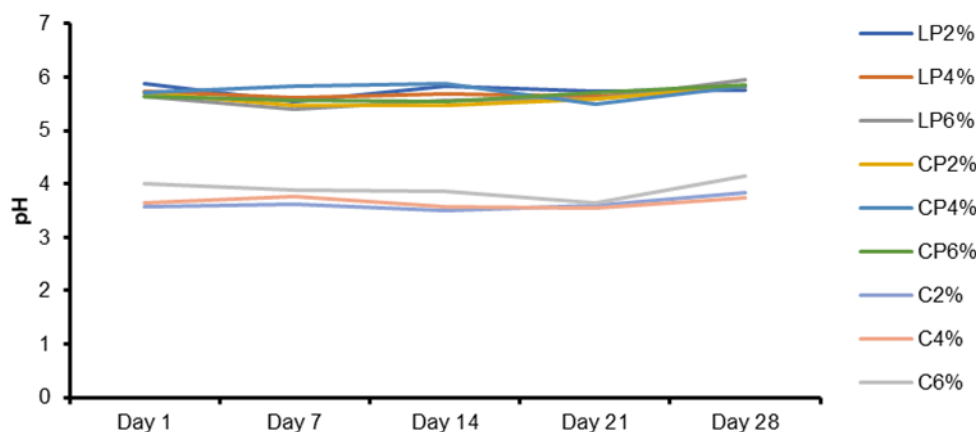


Fig 4. The pH value of the blushes at 30 °C

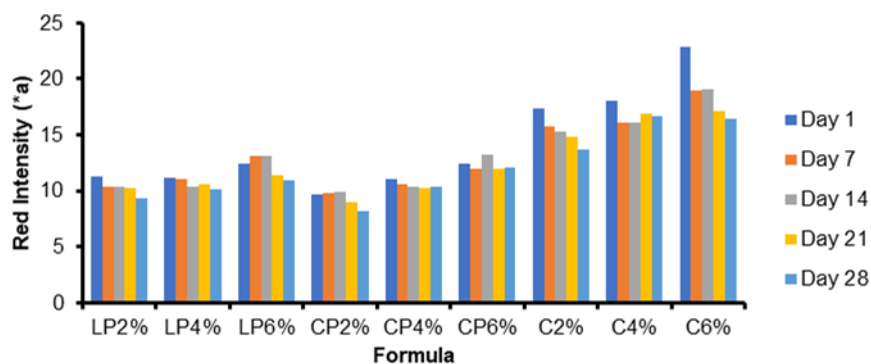


Fig 5. The stability of the blushes at 8 °C

to yellowish-red. This change might occur due to the breaking of bonds in betacyanin which causes a reduction in red color to pale red or turns bright yellow [34]. The color change could be caused by the pH change. Decreasing of pH would result in a change of pigment color from red to purple, whereas increasing of pH will turn it to yellow-brown [32]. As for the loose and compact powder preparation, discoloration occurred on the 14<sup>th</sup> and 21<sup>st</sup> day, respectively.

The discoloration occurred because the beetroot extract was easily oxidized [35] and the ascorbic acid content added in it as an antioxidant was not strong enough to protect the dosage forms. The addition of ascorbic acid was used to maintain the color stability of the extract from the influence of air because the ascorbic acid will be oxidized first so that the oxidation of the

extract can be avoided [36]. The cream was oxidized earliest because of the water content. Water content could accelerate the oxidation process so that the dosage form was not stable enough. The compact powder was oxidized longer because the surface area was smaller than the loose powder. The compact powder stuck together to form a compact solid powder and reduced the air which came into contact with the dosage form.

At 8 °C (Fig. 6), the three dosage forms (compact powder, loose powder, and cream) showed fair stability. It was evident that on the 28<sup>th</sup> day, each dosage form still had red color. Therefore, the color stability was greatly influenced by the storage temperature. According to the previous study [36], betacyanin which acts as a dye in beetroot extract was stable at temperatures under 40 °C.



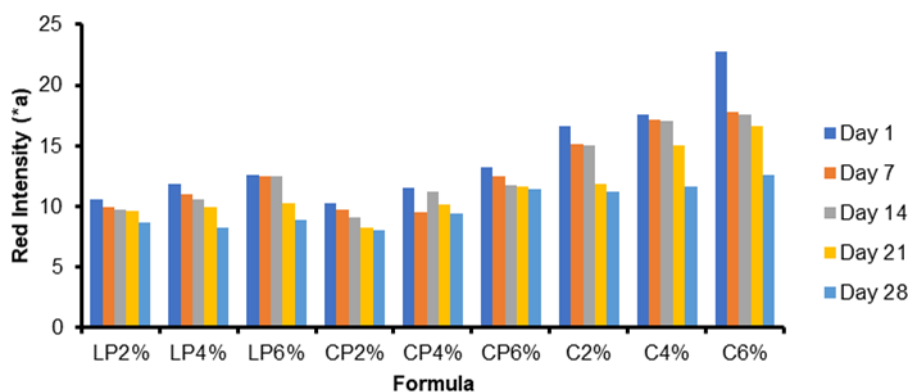


Fig 6. The stability test of the blushes at 30 °C

### Hedonic Test

The hedonic test was only performed on compact powder because it was the most stable dosage compared to the cream and loose powder. The sensory analysis has been used because it can guide the process of research and development of a cosmetic product. This analysis allows researchers to identify the acceptance level of users about the product [37]. This analysis method is subjective and is used to evaluate a product using human senses. This method was used in this research to determine whether the preparation of a cosmetic formula in compact powder form was considered pleasurable based on a hedonic scale using a preference scale points of 9 indicating "extremely like" and 1 "extremely dislike" [37]. The hedonic test on

this study was approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia (Reg. number: KET.847/UN2.FI/ETIK/PPM.00.02/2019). The hedonic test for the color and appearance of the compact powder (Fig. 7) showed that the extract concentration of 2% had lower value with an average value of 6 (fairly like) compared to the concentration of 4% with an average value of 7 (like) and the concentration of 6% with an average value of 8 (really like). In the case of taste/sensation and odor, the average value of the three concentrations were the same indicating that all panellists liked it. The hedonic test performed on all formulas of the compact powder dosage form showed that the most preferred blush was in the formula with a concentration of 6%.

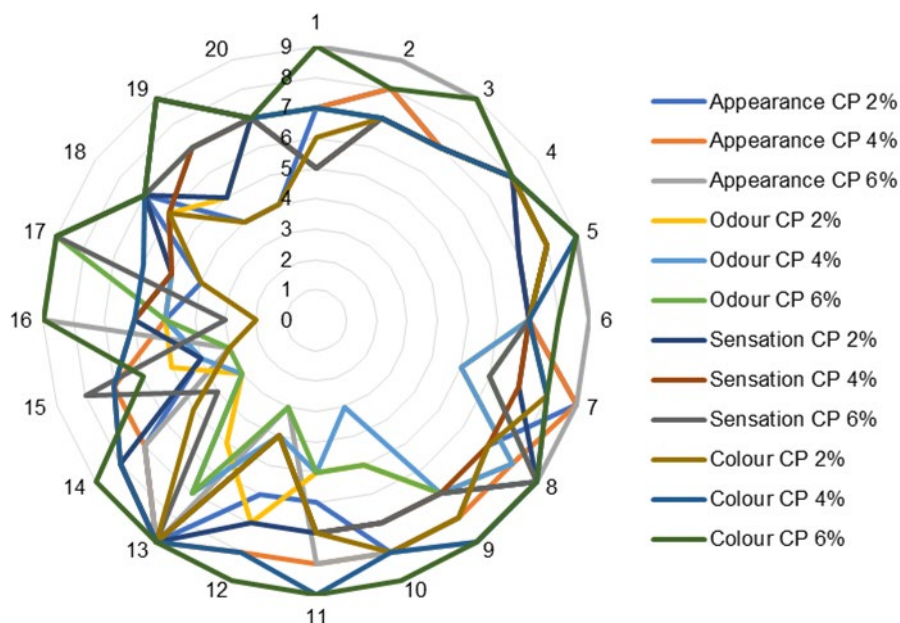


Fig 7. The hedonic test results, see Table 2 for the hedonic scale

## ■ CONCLUSION

Beetroot (*Beta vulgaris* L.) extract might contain flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids. The dry extract of beetroot (*Beta vulgaris* L.) can be formulated into blush dosage forms. The color stability (\*a) value of each dosage form decreased during 28 days of storage. However, discoloration of \*a that occurred in the compact powder was insignificant, which indicated that the compact powder was quite stable compared to loose powder and cream dosage forms. Therefore, we conclude that the compact powder was the most stable dosage form. All dosage forms were homogeneous and could be easily applied, and especially for the compact powder, there were no fractures that occurred after the breakage testing, and the pH remained stable for all formulas (between 3–5) after 28 days of storage. The most favored formula of the compact powder was the formula of 6% because it produced color when applied to the skin.

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