

Antioxidant and Antibacterial Activity of Pomegranate Extract (*Punica granatum* L.) in Lip Balm Formulation

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Abstract

Lips are a part of the face that does not have hair follicles making them easily dry and crack. Lipbalm is a beauty product that could be used to solve this problem by increasing lip moisture. However, the synthetic ingredient in commercial lip balm can have side effects on the body in the long term. Pomegranate fruit is one of the natural ingredients containing anthocyanin which can be used as a natural dye, antioxidant, and antibacterial. Therefore, this study was performed to study the potency of pomegranates as natural dyes, antioxidants, and antibacterial in lip balm. Pure pomegranate juice was obtained through squeeze step. Lip balm was prepared in several concentrations of pomegranate juice which were 0%, 12.5%, 18.75%, and 25%. Phytochemical screening shows the content of anthocyanin, saponin, tannin, and flavonoid. In this study, pomegranate juice has low antioxidant activity with IC₅₀ 449 ppm. Lip balm formula with 18.75% and 25% of pomegranate juice can inhibit the growth of the bacteria *Staphylococcus aureus*.

Keywords: Antibacterial; Antioxidants; Lip balm; Lips; Synthetic Ingredients.

INTRODUCTION

One part of the face that most influences the aesthetic perception of the face is the lips. According to Mulyawan and Suriana (2016) in Nazliniwyaty et al., (2019) the most sensitive and unprotected part of the face is the lips. Lips often look dry and cracked when it is exposed to cold air or excessive heat so that they are unsightly. This is because the lip does not have sweat glands and hair follicles that can protect it in the environment (Trookman *et al.*, 2009, Yayang *et al.*, 2019). One of the cosmetics products that can overcome this problem is lip balm.

Lip balm is a preparation that is applied to the lips and functions as a moisturizer by forming an immiscible oil layer on the surface of the lips. The layer formed by the lip balm is a protective layer of the lips from the influence of the external environment. In addition to lipstick, lip cosmetics that are often used by women are lip balms. The purpose of lip balm is to increase moisture in the lips. The main components in lip balms are waxes, oils, and fats which aim to prevent dry lips from occurring by keeping the lips moist and protecting the lips from environments that are too hot and too cold (Agustiana *et al.*, 2019).

Lip balm also contains antioxidants, preservatives, and coloring compounds. Antioxidants in lip balm function to prevent free radicals that are harmful to the skin. Preservatives are used to prevent irritation and

prevent bacterial growth, while the dye in lip balm is added only to make the product (lip balm) look attractive (Rini, 2012). Antioxidants that are often used in the manufacture of lip balms are BHT (butylated hydroxytoluene) and other preservatives.

Pomegranate is a tree that has a height of 2 to 5 meters. The pomegranate tree has a single leaf opposite each other (scattered). The large pomegranate flowers have red and white colors. The Pomegranate flower is a pansy flower, and separated. The flower axis is hollow with a conical shape, 5 to 7 corollas in irregular buds, and many stamens, free stamens, and ovary sink. While for the pomegranate itself has a size 5 inches with red and dark red skin and is shaped like a grenade. The taste of the pomegranate itself varies depending on the level of maturity of the pomegranate (Sharrif and Hamed, 2012).

Pomegranate fruit, also known as Pomegranate fruit, has several active compounds, namely alkaloids, flavonoids, saponins, tannins, and triterpenoids. Pomegranate contains two types of polyphenol components, namely flavonoids (anthocyanins) and hydrolyzed tannins which have antioxidant activity. The antioxidant activity of pomegranate is higher than the antioxidant activity of red wine and green tea. In addition, the antioxidants in pomegranate also have the potential to reduce lipid peroxidation (Roswiem *et al.*, 2014).

Therefore, in this research, researchers would make a lip balm made from natural ingredients. One of the natural ingredients used in this research is pomegranate. The use of natural ingredients aims to reduce and replace synthetic ingredients that exist in lip balm preparations. The compounds in the pomegranate (*Punica granatum* L.) to be tested are anthocyanins, saponins, tannins, and flavonoids, each of which has a role to replace or reduce synthetic ingredients commonly used in the manufacture of lip balms, so that in the manufacture of lip balms from natural ingredients is expected to bring out the natural color of the pomegranate, as well as antioxidants and antibacterial from the natural ingredients used.

MATERIALS AND METHODS

This study uses Petri dishes, beakers (pyrex), object glassed, measuring cups, oven (Memmert), microscopes, test tubes, LAF (Laminar Air Flow), micropipettes, stoves, UV-Vis spectrophotometers (Genesys 10) as an antioxidant test and testing the total phenolic content, filter cloth, pH meter, oven, knife, and lip balm container, while for the used materials is a pomegranate from a fruit shop in Yogyakarta, olive oil, beeswax, oleum cacao (Kimia Jaya Labora), glycerin, aquadest, cetyl alcohol, DPPH solution, methanol pa, HCl 2N solution, FeCl₃ solution, Folin ciocalteau reagents, Na₂CO₃ solution, NaOH solution, and *Staphylococcus aureus* bacteria culture. In sample preparation, the used fresh pomegranate is ± 1 week after harvest that has red color. Three kilograms of pomegranate fruit is separated between the skin and flesh, then the flesh is washed until clean and weighed (Yuska *et al.*, 2018).

Procedures

Extraction

The extraction process was carried out by squeezing the pomegranate and filtering the pomegranate to get pure juice using a white filter cloth. The filtering process was carried out by squeezing the pomegranate which is still together with the seeds then placed on a filter cloth and then the juice was put into a 1000 mL sterilized beaker glass. Yield is calculated using this formula:

$$\text{yield extract} = \frac{\text{weight of juice}}{\text{weight of initial simplicia}} \times 100\%$$

Phytochemical Screening of Pomegranate Juice

Anthocyanins

The color test of anthocyanin compounds was carried out according to Harbone (1987). Red pomegranate juice 0.5 g was added to 2M HCl in 2 drops and then heated at 100°C for 5 minutes. A positive result is indicated with a red color. The several dropwise of 2M NaOH was added while observing the color changes.

Positive result is indicated by the changes of red color to a blue-green color which then fades slowly (Putri Ni Ketut *et al.*, 2015).

Saponin

The Red Pomegranate juice was weighed as much as 0.3 g and then put into a test tube. Then 5 mL of distilled water was added and shaken for 30 seconds. The positive result is marked by the formation of foam which is stable for 30 seconds on the surface of the solution.

Tannin

The red pomegranate extract 1 g was added to 10 mL of distilled water and then boiled. After cooling, 5 mL of 1% FeCl₃ was added. If the color changes to dark blue, then the sample contains tannins (Novilia, 2014).

Flavonoid

The extracted sample 1 g was put into a test tube. Then 3 drops of concentrated HCl were added and then heated for 15 minutes in a water bath. If red or yellow color is formed, it means that the juice contains flavonoids (flavones chalcones and aurons) (Muthmainnah, 2017).

Determination of total phenol content

A total of 2 mg of pomegranate juice was dissolved in 2 mL of methanol and homogenized. Then 0.2 mL of extract was put into a test tube and added 1.8 mL of distilled water and 0.1 mL of Folin Ciocalteu reagent. The solution was homogenized, vortex and incubated for 5 minutes. Next, 1 mL of 5% Na₂CO₃ was added and homogenized. Distilled water 1.9 mL was added to obtain final volume of 5 mL. Then solution was homogenized and incubated for 5 minutes. The absorbance was measured at a wavelength of 760 nm. Gallic acid was used as a standard curve with a concentration of 12,5; 25; 50;100 and 200 ppm. A linear equation is obtained by plotting a linear graph between the gallic acid concentration (x) and the absorbance value (y) (Yismairai *et al.*, 2019). Calculation of total phenolic content is performed using the formula below:

$$TPC = \frac{C \times V \times fp}{m}$$

Annotation:

C : fenolic concentration (x score)

V : volume of extract used (mL)

fp : Dilution Factor

g : Weight of sample used (g)

TPC : Total Phenolic Content (mg GAE/ g sample)

Antioxidant Activity Test

Antioxidant activity was determined by the DPPH free radical method according to Santosa *et al.* (1998). In this test, the sample of pomegranate juice were made in several concentrations which are 200,400, 600, 800, and 1000 ppm. The concentration of DPPH is 10 ppm. The

stock solution was 1000 ppm. The DPPH solution was put into each tube with various concentrations, namely 2 mL of DPPH 10 ppm + 1 mL of sample solution, while for the control, 1 mL of DPPH 10 ppm was made, then incubated for 30 minutes in the dark room temperature, then the absorbance was measured at a wavelength of 517 nm. Furthermore, the value of inhibition concentration 50% (IC₅₀) was determined, namely the concentration of the sample that can reduce DPPH free radicals by 50% by using the equation $y = ax + b$, thus obtaining IC₅₀ in units of g/ml. (Abdullah *et al.*, 2014).

Extract Dosage Formulation

In this study, four lip balm formulations were made, consist of 0, 12.5, 18.7, and 25% of pomegranate extract with a weight of 12.8 g each container. The composition of the pomegranate extract lip balm is presented in Table 1.

Table 1. Formulation of Natural Lip balm.

Ingredient	Red Pomegranate Extract Formulation (%)			
	F0	F1	F2	F3
Red Pomegranate Extract	0	12.5	18.75	25
Olive Oil	10	10	10	10
Beeswax	25	25	25	25
Glycerin	10	10	10	10
Cetyl alcohol	50	50	50	50
Oleum cacao	Ad 100	Ad 100	Ad 100	Ad 100

Annotations:

F0 = Lip balm formula as a base

F1 = Lip balm formula with concentration extract 12.5%

F2 = Lip balm formula with concentration extract 18.75%

F3 = Lip balm formula with concentration extract 25%

In the process of making lip balm, the first step was to put ethyl alcohol in a beaker glass, heated and added pomegranate juice slowly. The solution was stirred until evenly mixed between ethyl alcohol and extract. After that, beeswax and cacao oleum was added and melted a temperature of 65°C. Next, olive oil was added, heated, and stirred until homogeneous. Lastly, the solution was taken out from water bath and poured into a container lip balm mold evenly and cooled until it hardens at room temperature (Dirjen POM, 1985).

Organoleptic Test

An organoleptic test was carried out using the five senses. The parameters include odor, color, and texture of lip balm (Yulyuswarni, 2018). Evaluation of the preparation was carried out for 3 weeks with inspections carried out every week (days 1, 5, 10 and so on).

Homogeneity Test

This test was carried out by testing the homogeneity of the preparation of each lip balm preparation by taking the lip balm preparation and placing it on the glass slide

and then visually observing whether there were coarse grains or not (Keithler, 1956).

Lip balm Weight Test

This test was carried out by weighing the lip balm preparation including the container. This weighing was carried out every 7 days for 3 weeks at a closed room temperature of 27°C. This test was carried out to determine the increase or decrease in weight at the beginning of manufacture and during storage.

Melting Point Test

This test was done by melting 1 g of the lip balm preparation in a beaker. The melting temperature was measured at the initial melting (Dian, 2019).

pH Test

pH value was measured using a pH meter where 1 g of the sample was inserted and then dissolved in 10 mL of distilled water, after that electrode is inserted or dipped in the lip balm solution, then the numbers show on the pH meter are seen (Risnawati *et al.*, 2012).

Irritation Test

This test was carried out to see and evaluate whether the product can cause irritation or not. The technique used in this irritation test is an open patch test on the forearms of 30 panelists. An open patch test was carried out by applying the prepared preparation at the location of the attachment, leaving it open and, observing what happens. Reaction was observed. Positive irritation reactions are characterized by redness, itching, or swelling (Risnawati *et al.*, 2012).

Favorite Test

This test was conducted to determine the level of panelist preference. The age of 30 female panelists was around 18-22 year old. The panelist was not having sensitive or allergic skin. Each other panelist was asked to apply lip balm of various concentrations of red pomegranate to the back of the hand. Then they were asked to fill out the provided questionnaire. The duration of trial was ± 15 minutes and after trying the lip balm, the panelists are expected to wash their hands.

Antibacterial test

Antibacterial activity test was done using pour plate method. Bacterial incubation was carried out for 24 hours using the pour plate method at 37°C. Then diameter of the inhibition zone was measured for each sample. The list of samples is presented in Table 2.

Table 2. List of Samples.

No	Code	Sample
1	E	Pomegranate Extract with Concentration 12.5%, 18.75% dan 25%
2	B	Olive Oil, beeswax, oleum cacao, glycerin dan cetyl alcohol
3	F1	Lipbalm formula with concentration extract 12.5%
4	F2	Lipbalm formula with concentration extract 18.75%
5	F3	Lipbalm formula with concentration extract 25%
6	K	Commercial Lipbalm

Antibacterial test was performed to determine the ability of antibacterial activity against *Staphylococcus aureus*. This first step is to culture the bacteria on Nutrient Agar (NA) and Nutrient Broth (NB) medium. Nutrient Agar (NA) is a medium for rejuvenating *Staphylococcus aureus* which is in solid form, while Nutrient Broth (NB) has same function as (NA) for growing bacteria but in liquid form. Then, 100 μ L Nutrient Broth (NB) containing bacteria was poured aseptically to each petri disk. MHA media 20 mL was added into each petri disk. This step can also be performed by formerly mixing the bacteria and MHA media, then small holes are made after the media hardens to put the sample. Plates were divided into 3 to 4 zones to facilitate the distribution of treatments. After samples had been put into all petris, each petri was wrapped and incubated at 37°C for 24 hours under aerobic conditions for *Staphylococcus aureus*. The ability of antibacterial activity was shown through the inhibition zone. The diameter of the finhibition zone was measured using a ruler.

RESULTS AND DISCUSSION

Secondary Metabolites of Pomegranate Juice

The yield of pomegranate juice is 76.80% (1.565 kg of pomegranate juice from 2.038 kg of peeled pomegranate) by squeezing the pomegranate. Dian (2019) also carried out this method on dragon fruit with the similar yield as this study. Phytochemical test results from pomegranate juice is presented in Table 3.

Table 3. Phytochemical Screening Data of Pomegranate Juice.

Compound	Parameter	Analysis Result	Description
Anthocyanin	Blue Green	+	Green
Saponin	Stable Foam	+	Presence of Foam
Tannin	Dark Blue	+	Dark Blue
Flavonoids	Red/Yellow	+	Red

Annotation: (+) there are chemical compounds, (-) No chemical compounds

In this study, pomegranate extract showed a positive result for the anthocyanin test, namely a change in color to green. This pigment belongs to the flavonoid

compound and is responsible for the appearance of red, orange, blue and purple colors in some leaves, fruits, and flowers. Anthocyanins have potential natural dyes to replace synthetic dyes (Gross, 1987).

Saponin is also detected in the pomegranate juice. Saponin compounds are included in triterpenoids, usually found in higher plants. Saponins are abundant in leaves and roots but are rarely found in fruit (Cseke *et al.*, 2006). Saponin compounds are indicated by the presence of stable foam or foam.

The next compound identified was Tannin. Tannin compounds have positive results by showing a dark blue color. Tannins are a class of polyphenolic compounds that are also commonly found in plants. Tannins can be defined as polyphenolic compounds with very large molecular weights, which are more than 1000 g/mol, and can form complex compounds with proteins, besides that, tannins have a major biological role because of their function as protein precipitants and metal chelators. It also acts as a biological antioxidant. (Noer *et al.*, 2018)

The last test of flavonoid compounds in this research showed positive results where the color produced was red. Flavonoids are secondary metabolites of polyphenols, which are found widely in plants and food and have various bioactive effects including anti-viral and anti-inflammatory (Qinghu Wang *et al.*, 2016). Flavonoids are also one of the natural compounds that are commonly found in plants and food to treat various diseases such as cancer, antioxidants, bacterial pathogens, inflammation, cardiovascular malfunction, and have antioxidant abilities in preventing injury caused by free radicals (Arifin *et al.*, 2018)

Total Phenolic Content in Pomegranate Extract

This research used gallic acid as a standard according to Singleton *et al.* (1999). The standard curve of gallic acid is presented in Figure 1. Gallic acid has been used as a standard of comparison which is stated as milligrams of gallic acid equivalent per gram or milliliters of extract between samples (Tapera *et al.*, 2019). The gallic acid standard is the relationship between gallic acid concentration (x) and absorbance value (y), which this relationship will produce a linear regression equation $y = 0.0026x - 0.0116$ and R^2 value is 0.9903. From the equation in Figure 1, it was carried out to calculate the total phenol in the pomegranate extract. Total phenol levels are expressed in units of mg gallic acid equivalent/g sample, % Gallic Acid Equivalent (GAE) (mg GAE/g) (Hala *et al.*, 2020). These results indicate that the total phenol content in the pomegranate extract is 0.012 mg/mg GAE.

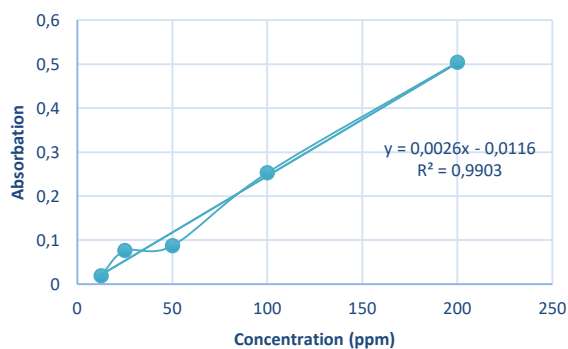


Figure 1. Gallic Acid Standard Curve.

Pomegranate Extract Antioxidant Activity

The result of antioxidant measurements on pomegranate extract can be seen in Figure 2. Based on the data in Figure 2, the results of linear regression are obtained, namely $y = 0.0649x + 20.805$ with a relation coefficient $R^2 = 0.924$ so that the IC₅₀ can be determined. The IC₅₀ value is the concentration of a test sample solution that provides 50% DPPH reduction (Molyneux, 2004). From the equation data, it was found that IC₅₀ in pomegranate extract had a value of 449 ppm.

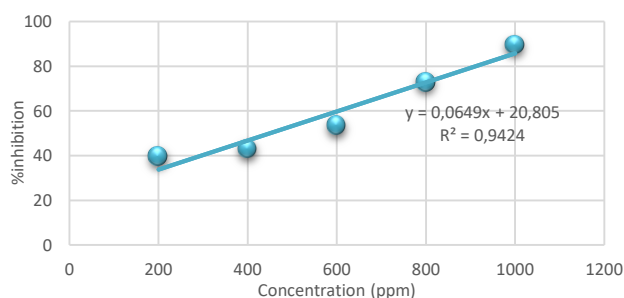


Figure 2. Antioxidant Activity of Pomegranate Extract.

According to Molyneux (2004), the antioxidant activity in a compound is said to be very strong if the IC₅₀ value is <50ppm, strong if it has an IC₅₀ value between 50-100 ppm, moderate if IC₅₀ value around 100-150 ppm and is said to be weak if the IC₅₀ value 151-200 ppm. The IC₅₀ value obtained from this research is 449 ppm, which is a weak IC₅₀ value. In Wulandari *et al.* (2017), IC₅₀ of pomegranate peel extract with the extraction process, namely maceration, got a value of 2.39 ppm, which means the IC₅₀ is very strong. The low IC₅₀ of pomegranate extract in this study is probably caused by the difference of extraction process.

Preparation of Pomegranate Extract Lip balm

Lip balm preparation from Pomegranate extract is pink. The concentrations used were 0%, 12.5%, 18.75%, and 25%. Figure 3 shows that the higher concentration, the

more concentrated the color will be. The result of the lip balm preparation can be seen in Figure 3.

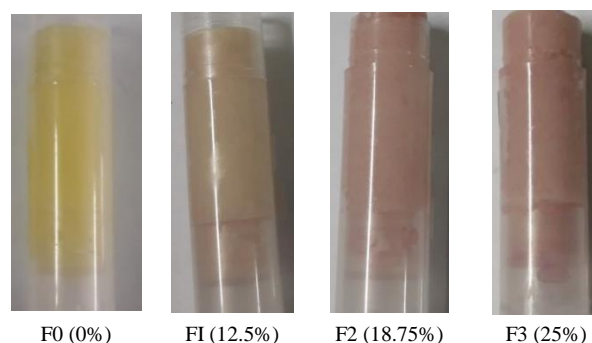


Figure 3. Lip balm from Pomegranate Extract in Several Concentrations.

Organoleptic Test Results

Lip balm preparations from various extract concentrations 0%, 12.5%, 18.75%, 25%, had different characteristics of color, smell, and texture. The organoleptic test aims to determine the stability conditions of each concentration of lip balm tested for 3 weeks. It is included color, odor, and texture of each concentration of lip balm.

Based on organoleptic data for 3 weeks, changes in texture, aroma and color began to occur in the second week. The organoleptic test is conducted by Dian (2019) also showed changes in the second week. According to Dian (2019), physical differences (color and texture) are caused by a high room temperature factor which cause faster water evaporation so the particles in the lip balm clump together and cause the formation of crystals. According to Pertiwi *et al.*, (2020), the higher the concentration of extract used in the lip balm formulation, the stronger the color intensity.

Homogeneity Test Results

Based on homogeneity data, at concentrations of 0%, 12.5%, 18.75%, and 25% of the lip balm were homogeneous, while for a concentration of 12.5%, the results were not homogeneous. The homogeneity data showed that all lip balm formulations did not show any coarse granules, while the concentration of 12.5% was not mixed evenly for color distribution. Therefore, it can be said that the preparations are 0%, 18.75%, and 25% and have a homogeneous composition in which the color distribution is even and there are no coarse grains on the glass object. This is in line with research conducted by (Yusuf, 2019)

pH Test Results

Testing the pH of the pomegranate extract lip balm in this study was using a pH meter. The purpose of pH testing is to determine and obtain a pH value that is close to the physiological pH of the skin, namely 4.5-6.5. The results of the pH test can be seen in Table 4.

Table 4. pH lip balm from Pomegranate Juice.

Concentration	pH
F0 (0%)	5.41
F1 (12.5%)	4.61
F2 (18.75%)	4.24
F3 (25%)	4.01

According to Table 4, pH value of the lip balm without extract (F0) is 5.41, the concentration of F1 (12.5%) containing pomegranate extract is 4.61, then for the concentration of lip balm using pomegranate extract F2 (18.75%) was 4.24 and the concentration of F3 (25%) had a pH of 4.01. The difference in pH was caused by differences in the concentration of the extract. At concentrations of 0% (F0) and 12.5% (F1) it is already in the vulnerable pH of the lip skin (Tranggono and Latifah, 2007) which says the physiological pH of the lip skin is between 4.5-6.5, which means lip balm from extracts. Pomegranate is safe to use, while the concentrations of 18.75% and 25% do not meet the physiological pH requirements of the lip skin. This is because the amount of pomegranate extract that is included in the lip balm preparation during the manufacturing process, so that the more concentrations used in the preparations, the more acidic it will be because the pH of the pomegranate is acidic, which 3.51.

Melting Point Test Results

In this research, the melting point test was also carried out. A melting point test is done to find out at what temperature the lip balm can melt completely. The result data are presented in Table 5.

Table 5. Lip balm Melting point from Pomegranate Extract.

Concentration	Melting Point (°C)
F0 (0%)	55
F1 (12,5%)	53
F2 (18,75%)	52
F3 (25%)	52

From the results of the melting point test on the lip balm preparation, F0 (0%) has a melting point of 55°C, F1 (12.5%) has a melting point of 53°C, then for F2 (18.75%) is 52°C and the last one for F3 (25%) which is 52°C. All melting point results have been included in the standard based on SNI 16-5769-1998 in Ratih (2014) which is in the range of 50-70°C. It can be said that the melting point of the prepared lip balm has good melting point and is up to standard. According to (Agustiana *et al.*, 2019) the difference in melting point in each preparation is due to the difference in the amount of cacao oleum included in each lip balm, the higher the

concentration, the less cacao oleum is added so that the resulting melting point decreases. Data shows that F0 (0%) has the highest melting point which is caused by the amount of cacao oleum that is the most compared to other concentrations is 5,2 g, while the least amount of cacao oleum is in the concentration of F3 (25%) which is 2 g, so it has a lower melting point.

Lip balm Weight Test Result

The result of the observed lip balm weight for 3 weeks is presented in Table 6.

Table 6. Lip balm Weight Test Data.

Week	Weight (g)			
	F0 (0%)	F1 (12.5%)	F2 (18.75%)	F3 (25%)
0				
Average	6.207	6.695	6.871	6.697
1				
Average	6.207	6.693	6.868	6.695
2				
Average	6.208	6.692	6.861	6.655
3				
Average	6.210	6.692	6.859	6.644

Lip balm weight test data for all concentrations were included in the Paired Samples Test using SPSS to determine whether there was a difference in lip balm weight. Tests using Paired Sample Test are seen based on the comparison of week 0 and week 1, week 1 and week 2, week 2 and week 3, and the last week 0 and week 3. Based on the results of the Paired Samples Test, there was no significant difference in the weight of the lip balm which was weighed every week. If the sig value >0.05 then there is no significant difference in the lip balm weight test which is carried out every week.

Favorite Test Results

In this research, the preference test was carried out by applying the four preparations of pomegranate extract lip balm (F0, F1, F2, F3) to 30 panelists, then the panelists were asked to fill out a questionnaire to find out which lip balm preparation was the most attractive according to the panelists. Based on the test result, it was found that those who liked the lip balm preparation for the texture were 63.3% for the 25% formulation, the panelists who liked the lip balm preparation for the aroma were 76.19% for the 25% formulation, while the largest color was 77.4% for the concentration of 25% and overall including texture, aroma and color is 67.7% for a concentration of 25% as well. Based on the lip balm preference test with aroma texture and color parameters, the lip balm most preferred by the panelists was lip balm with a concentration of 25%. The 25% concentration is the lip balm concentration with the most pomegranate extract compared to other concentrations.

Irritation Test Result

The result of the irritation test is shown in Table 7.

Table 7. Irritation Test Result Data on Pomegranate Lip balm.

Irritation Test	F0 (0%)	F1 (12.5%)	F2 (18.75%)	F3 (25%)	Number of Respondent
Itchy Rash	0	0	0	0	0
Redness	0	0	0	0	0
Bump	0	0	0	0	0
Other irritation	0	0	0	0	0

Based on the results of the tests carried out, the panelists gave negative results or there were no signs indicating irritation such as the absence of red skin, itching, or the presence of swelling and other allergic reactions. This shows that the ingredients contained in the lip balm from pomegranate extract are not irritating. This is in accordance with research (Nazliniwaty *et al.*, 2019) that lip balm from pomegranate extract does not cause irritation. The conclusion that can be drawn is that all the lip balm concentrations tested did not cause irritation.

Antibacterial Test Results

In this research, antibacterial testing was carried out to determine the level of antibacterial in the sample to be tested against *Staphylococcus aureus* bacteria which is a gram-positive bacterium that grows at an optimum temperature of 37°C, this bacterium that is usually present on the skin, digestive tract, and intestines. Human respiratory tract can be found on the lips of humans (Kusuma, 2009).

This antibacterial test used the pour plate method which was repeated 3 times for each sample tested to determine the inhibition zone formed in the sample tested using *Staphylococcus aureus* bacteria. The result of antibacterial testing can be seen in Table 8.

Table 8. Inhibition Zone Measurement Data.

Sample	Average Diameter of Inhibition Zone (mm)
Extract 12.5%	13.33 ^{a,b,c}
Extract 18.75%	14.66 ^{b,c}
Extract 25%	22.66 ^c
Lipbalm 12.5%	0 ^a
Lipbalm 18.75%	5.33 ^{a,b}
Lipbalm 25%	6 ^{a,b}
Control + (ciprofloxacin)	40.94 ^d
Control – (aquadest)	0 ^a

Description:

a: mean are included in subset a and have no significant difference

b: mean are included in subset b and have no significant difference

c: mean are included in subset c and have no significant difference

d: mean are included in subset d and have no significant difference

The presence of an inhibitory zone in the pomegranate extract was due to the pomegranate extract containing antibacterial compounds (saponins) so that it

could inhibit the *Staphylococcus aureus* bacteria. In this research, the three extracts (12.5%, 18.75% and 25%) had an inhibitory zone with a vulnerability of 11-20 mm which means strong, while for positive control it had an inhibition zone of 21 so it was very strong (Yunus *et al.*, 2018). The inhibition zones of the pomegranate extract lip balm formed were 18.75% and 25% concentrations, only in the first repetition. The lip balm inhibition zones with concentrations of 18.75% and 25% had a strong inhibitory power with a range of 21 (Yunus *et al.*, 2018). One of the constituent materials formed by the inhibition zone is glycerin, where glycerin has a very strong inhibitory zone strength 21 as well as a positive control (Yunus *et al.*, 2018).

The inhibitory zone data for the antibacterial test in this research were then entered into the ANOVA test using SPSS. The ANOVA test was used to determine whether or not there was a difference in the sample with an inhibitory zone. The ANOVA results showed a significant difference in the inhibition zone formed in the pomegranate extract and lip balm preparations from the pomegranate extract because $000 < 0.05$ so H_0 was rejected. And H_1 is accepted.

The results of the subset table are then entered into Table 8. Table 8 column 1 (a) shows that F1, F2, F3 lip balm, and 12.5% extract have similarities and there are no differences. Table subset 2 (b) shows that lip balm F2, F3 extract 12.5% and 18.75% there is no difference, different from the table for subset 1 (a) earlier, next is table subset 3 (c) where extract 12.5%, 18.75%, and 25% have no significant difference, in contrast to table subset 2 (b), that table subset 2 has similarities with lip balm F2 and F3. The last table subset 4 (d) is the positive control which has differences with pomegranate extract and lip balm preparations because it has the greatest value among the others. From the results of the antibacterial test in Table 8 and the test using SPSS the largest diameter of the inhibition zone was pomegranate extract with a concentration of 25% and the lowest was lip balm with a concentration of 12.5%.

CONCLUSIONS

The ability of pomegranate extract as an antioxidant is 449 ppm, which in pomegranate extract contains

antioxidants, but based on IC50 it is very low. The ability of pomegranate extract as an antibacterial in inhibiting the growth of *Staphylococcus aureus* bacteria was found in the three extract concentrations (12.5%, 18.75% and 25%) while in lip balm preparations there were concentrations of 18.75% (F2) and 25% (F3), while the ability of pomegranate extract as a colorant in lip balm preparations can last for 3 weeks at room temperature and the lip balm which produces the brightest color is a concentration of 25% (F3). This concentration is also the most preferred by the panelists.

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