

Formulation and Antioxidant Activity of Lip Balm Prepare Enriched by Bidara Leaf Extract (*Ziziphus spina-christi* L.)

Hendrawati*, Siti Aidina, Maulidia, Nurhasni, and Yuliani Nur Azizah

Faculty of Science and Technology, State Islamic University Syarif Hidayatullah Jakarta, Indonesia

*Corresponding Author: hendrawati@uinjkt.ac.id

Received: January 23, 2022; Revised: February 28, 2022; Accepted: June 15, 2022

Abstract

Bidara leaves (*Ziziphus spina-christi* L.) are known for their high antioxidant activity. It can be used as an additional active component in the preparation of lip balm formula. The purpose of this research is to get lip balm formula enriched with Bidara leaf extract and determine its antioxidant activity. The antioxidant activity of Bidara leaf in ethanol extract was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the active component that gave the role to it was identified using GCMS (Gas Chromatography and Spectrometry Mass). The result showed that Bidara leaf extract contained a phytol component with highly strong antioxidant activity. The preparation formula of lip balm used 0, 1, 2 and 3% (w/w) of Bidara leaf extract expressed by F0, F1, F2, and F3 respectively. All lip balm products tested have met the requirements for Indonesian National Standard (SNI) 16-4769-1998 criteria encompass melting point range 53.50 – 54.50 °C; pH value 4.43 – 4.53; homogeneous; and negative microbial contamination. The most optimum Bidara lip balm product based on general panelists preference level was F1, which content 1% of Bidara leaf extract with half-max inhibitory concentration value (IC50) of 339.23 ppm.

Keywords: Antioxidant; Bidara leaf; Cosmetic; Lip balm; Indonesian National Standard

1. Introduction

Bidara leaves (*Ziziphus spina-christi* L.) or known as *Sidr* are very beneficial for the skin because they contain natural antioxidants (Elaloui *et al.*, 2016; Khaleel *et al.*, 2016). Antioxidants are inhibitors used to inhibit free radicals by forming a relatively stable reaction (Molyneux, 2004). The most relevance *in vitro* measurement used to evaluate the therapeutic potential of radical scavenger is half-maximal inhibitory concentration a.k.a. IC50. It indicates how much of a specific pharmacologic agent is required to inhibit free radical by half (Aykul and Martinez-Hackert, 2016).

Secondary metabolite compounds present in the content of a material are responsible for the antioxidant content of the material. Kusriani *et al.* (2015) determined the phenolic content of the ethanol extract of Bidara leaves by 7.19% and has antioxidant activity with

IC50 value of 127.87 ppm. The research was strengthened by Haeria (2016) which states that the ethanol extract of bidara leaves has a flavonoid content of 1.53% and antioxidant activity with an IC50 value 90.96 ppm. The ethanol extract of bidara leaves has been formulated in cosmetic preparations in the form of a peel-off-gel mask and has been tested to contain antioxidants (Hendrawati *et al.*, 2020). Based on the research sources above, Bidara leaves contain antioxidants that have the potential to be additional active ingredients in the manufacture of cosmetic preparations. In this study, Bidara leaf extract was added to lip balm cosmetics.

Lip balm is a transparent cosmetic preparation with main components such as wax, fat, and oil from natural or synthetic extracts to prevent dryness by increasing lip moisture and protecting the lips from

environmental adverse effects (Kwunsiriwong, 2016; Sulastomo, 2013). It is needed by many people to protect their lips from the sun and prevent lips disease (Tranggono and Latifah, 2007). Lips are part of the body that more delicate and sensitive than the other skin because they tend to be thinner and smoother (Wibowo, 2008). Sun exposure can cause free radical reactions that result in dried and cracked lips, darken lips, premature aging, and skin cancer (Draelos and Thaman, 2006). Therefore, using lip balm with some addition of antioxidants agent is highly suggested to protect the lips from free radical damages (Jacobsen *et al.*, 2011).

The formulation of lip balm cosmetics enriched with Bidara leaf extract has been carried out. Based on Tranggono and Latifah (2007), addictive substances that are non-toxic, non-allergenic, stable, and can be mixed with other ingredients in lip balm formulas, are needed to cover up the lack of lip balm. Bidara leaf is one of the local plants that are very beneficial for the skin because its extract contained antimicrobial, antifungal, anticancer, and antioxidant compounds (Kusriani *et al.*, 2015; Ashri, 2016; Putri, 2017), with quite low toxicity levels (Dhuha *et al.*, 2020). Therefore, Bidara can be used as a natural antioxidant to ward off free radicals and has the potential to be developed as a cosmetic, such as lip balm preparation.

Lip balm formulation was made with various concentrations of Bidara leaf extract as much as 0, 1, 2 and 3% (w/w), in cacao oleum base (Ratih *et al.*, 2014). The characteristics of lip balm were tested with the parameters of melting point value, pH value, homogeneity, and total microbial contamination following the quality requirements of Indonesian National Standard (SNI) 16-4769-1998. The SNI typically called standard quality for certain product that obtained by way of (third-party) product certification system to determine the conformity of a product with specified requirements through initial testing of samples of product in place to allow it to be sold anywhere in the Republic of Indonesia (BSN, 1998; Pundlik *et al.*, 2011; BSN, 2006). The preference level test was carried out on 30 untrained panelists and the result was analyzed statistically using one way, ANOVA

and Duncan test. The antioxidant activity of the best lip balm preparation was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and its stability was measured for 20 days to meet the quality standard (Mitsui, 1997).

2. Materials and Methods

The tools used in this study include lip balm containers, glassware (pyrex), analytical scales (Ohaus), magnetic stirrer, rotary evaporator (Heidolph-Lborota 4000), pH meter (Mettler Toledo), Perkin Elmer's Lambda 25 UV-Vis spectrophotometer, Gas Chromatography-Mass Spectroscopy (Shimadzu), melting point, blender and filter paper. The main ingredient in this research was Bidara leaf obtained from the Bidara garden in Sumenep (East Java, Indonesia) and has been determined at the Center for Biology for Botany, Biology Research Center-LIPI Cibinong. Technical ethanol was used as an extraction solvent. The test material for determining antioxidant activity used DPPH (Sigma) and methanol (p.a Merck). The ingredients in the lip balm preparation were cacao oleum, vaseline album (Brataco), glycerin (Moon K), sunflower seed oil (Mazola), and orange essence.

2.1 Extraction and phytochemical analysis of Bidara leaf

Bidara leaves that have been cleaned with water were dried in the air. The dried leaves samples were mashed to obtain Bidara leaves powder. The extraction process was carried out by soaking 200 g of Bidara leaves powder with 96% ethanol (v/v) until the sample was completely submerged, then stirred using a magnetic stirrer for 30 minutes and stored for 24 hours in a place protected from sunlight. The results of the Bidara powder immersion are filtered and then the solvent was evaporated using a vacuum rotary evaporator to obtain green concentrated Bidara leaf extract (Ashri, 2016). Afterward, the ethanol extract of Bidara was analyzed to examine steroid, triterpenoid, flavonoid, alkaloids, tannin, and saponin (Ikalinus *et al.*, 2015). The in vitro antioxidant activity of the extract was carried out using the DPPH

method (Goyal *et al.*, 2010) and the active compound was analyzed with GCMS (Kumar *et al.*, 2010).

2.2 Formulation and physical evaluation of Bidara leaf extract lip balm

The lip balm formulation process was carried out by melting cacao oleum, vaseline album, beeswax, and oil at 85 °C as mixture A, as represented at Table 1. Bidara leaf extract which has been added to glycerin is mashed with a mortar as mixture B. Mixture B was added to mixture A and then stirred until homogeneous. This mixing is carried out in a state of warm mixture A to minimize damage to the active substances in the Bidara leaf extract. The orange essence was added also to disguise the characteristic odor of the preparation.

Physical evaluation such as melting point, pH, smear test, homogeneity examination, and total plate number was carried out according to reference (BSN, 1998), where the number of microbial colonies were observed and calculated using equation 1:

$$AL = \Sigma C \times \frac{1}{df} \quad (1)$$

Where: AL = number of colonies,
C = number of colonies from each petri,
df = dilution factor

Stability checks of the lip balm formulation were carried out by storing the preparation at room temperature for 20 days. After 5 days, the preparation was observed for its changes in shape, color, and odor (Pundlik *et al.*, 2011).

2.3 Preference test for lip balm of Bidara leaf extract

Parameters of the preference test including texture, color, scent, homogeneity, and general preference were tested on the 4 lip balm formulations. The 30 untrained panelists were taken randomized with a rating scale presented in Table 4. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's test, when appropriate (BSN, 2006). Statistical significance was set at $P < 0.05$.

2.4 Antioxidant test of the extract and the lip balm preparation

The ethanol extraction of Bidara with different concentrations were put into test tube and mixed with 2 mL of DPPH 0.004 % (w/v). The mixtures were homogenized and incubated in the dark and room temperature for about 30 minutes. The absorbance of each solution was measured using spectrophotometer UV-Vis at 517 nm (Goyal *et al.*, 2010). Ascorbic acid was used as antioxidant standard in the range concentration of 0.5, 1, 2, 4, 6 and 8 ppm. The percentage of the DPPH radical scavenging is calculated using the equation 2 as given below:

$$\% \text{ inhibition of DPPH radical} = \left(\frac{A_{br} - A_{ar}}{A_{br}} \right) \times 100 \quad (2)$$

where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

Table 1. Lip balm formulation

Compositions	F0	F1	F2	F3
Bidara leaf extract (w/w %)	0	1	2	3
Sunflower seed oil (g)	1	1	1	1
Glycerin (g)	1	1	1	1
Beeswax (g)	3	3	3	3
Vaseline Album (g)	1.40	1.40	1.40	1.40
Orange essence (g)	0.40	0.40	0.40	0.40
Cacao Oleum (g)	Added until 20 g	Added until 20 g	Added until 20 g	Added until 20 g

Determination of antioxidant activity of lip balm prepare refers to previous research (Nurhaida *et al.*, 2017). The 1 gram lip balm samples were weighed and dissolved with 10 mL of methanol 98% (v/v). Furthermore, it was added with 20 mL n-hexane then extracted in separating funnel. Concentrated methanol phase was tested with the same procedure as the previous extract. The value of attenuation of free radical activity is expressed by IC₅₀ value. Concentration of samples or comparison antioxidants (ascorbic acid) and the percentage of inhibition is plotted respectively on the x and y axes in the equation of linear regression. Linear regression equation which is obtained in the form of the equation $y = a + bx$, used to find the value of IC₅₀ from each samples (Rahmayani, Pringgenies, and Djunaedi, 2013). The level of antioxidant power of the test compounds is classified according to Molyneux (2004).

3. Results and Discussion

Determination of leaf sample species was carried out at LIPI Cibinong showed that the type of Bidara leaf used in this study was *Ziziphus spina-christi* (L.) The extraction of Bidara leaves was carried out by the maceration method using ethanol. It was chosen as a solvent because of its neutral, good absorption, non-toxic, not easily overgrown by microbes and molds (Saadah and Nurhasnawati, 2015). The yield of Bidara leaf extract produced after being concentrated using a vacuum rotary evaporator was 16.3%. That result did not significantly different from previous studies (Hendrawati *et al.*, 2020). The extract obtained is thick in texture and dark green in color with a distinctive scent of Bidara leaf.

3.1 Phytochemical test of Bidara leaf extract

Phytochemical identification is a qualitative test to determine the presence of secondary metabolites contained in the extract. Based on phytochemical testing in Table 2, the sample of Bidara leaf was confirmed to contain compounds as listed below, that reinforced by the previous research (Hendrawati *et al.*, 2020). The results indicated that Bidara leaves have antioxidant activity because they showed positive results for the flavonoid, phenol, and tannin group test (Khanahmadi *et al.*, 2010; Salmiyah and Bahrudin, 2018).

3.2 Active compound of Bidara leaf extract using GCMS

Identification of active compounds using gas chromatography-mass spectrometry (GC-MS) was carried out to determine the active compounds contained in Bidara leaf extract. About 30 m long DB-5MS UI column was used as a stationary phase, with diameter and thickness film were 0.250 mm and 0.25 μ m, respectively. The results of the chromatogram are shown in Figure 1.

The results of GCMS analysis obtained 14 chemical compounds with different retention times, abundances, and peak segments. Based on the results of similarity with the literature, the 7th peak is the highest peak which is thought to be a phytol compound with a retention time of 32.471 minutes, a concentration of 21.98%, and a molecular weight (m/z) of 296. Bintoro, Ibrahim, and Situmeang (2017) stated that one of the phytochemical components in Bidara leaves is phytol compounds as well. The result was different as previously stated (Hendrawati *et al.*, 2020; Putri, 2017), that quercetin and rutin were found in the ethanol extract of Bidara leaves and acted as antioxidants.

Table 2. Phytochemical test result

Compound group	Discoloration	Conclusion
Steroids	Green	+
Flavonoids	Orange	+
Triterpenoids	Brown ring	+
Alkaloids	Chocolate	+
Phenolates	Black	+
Tannins	Blackish green	+
Saponins	Foaming	+

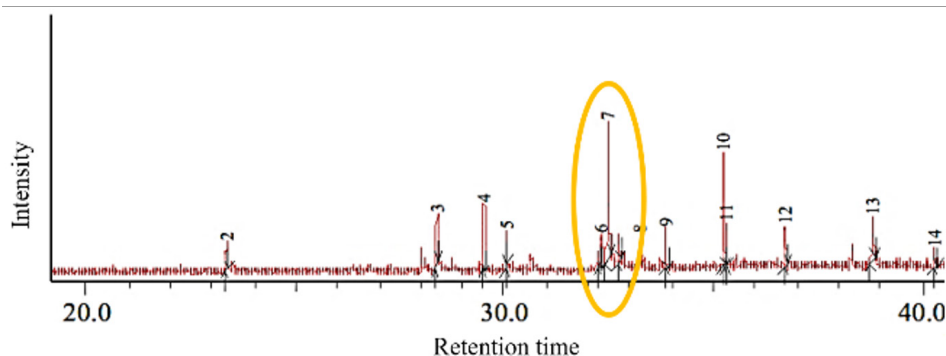


Figure 1. Bidara leaf ethanol extract chromatogram

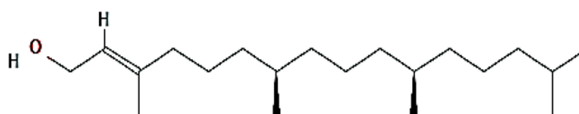


Figure 2. Structure of phytol

Phytol is an acyclic monounsaturated diterpene alcohol, present in vitamin K, vitamin E, and other tocopherols, which shown antioxidant activity (Moraes *et al.*, 2014). The branched-chain unsaturated alcohol and its antioxidant activity may be related to the hydroxyl group ($\text{OH}^\bullet$) present in its molecule, as depicted in Figure 2. Phytol reacts with a free radical, by donates hydrogen atoms with unpaired electron ($\text{H}^\bullet$), converting free radicals into less reactive species (Santos *et al.*, 2013).

3.3 Antioxidant activity of Bidara leaf extract

The in vitro antioxidant activity test of Bidara leaf extract (*Z. spina-christi* L.) was carried out using DPPH method. DPPH is characterized as a stable free radical reagent and mostly used to evaluate the antioxidant potential because it is furthermore rapid, simple and inexpensive in comparison to other antioxidant test models (Alam, Bristi, and Rafiquzzaman, 2020). The IC_{50} values obtained in Bidara leaf extract is 25.224 ppm (Table 3), that classified as highly strong antioxidant (Saadah and Nurhasnawati, 2015). The IC_{50} of 25.224 ppm means that the ethanol extract of Bidara leaf need as much 25.224 ppm to neutralize 50% free radical of DPPH. However, this result is still far below the ability of ascorbic acid as a standard

solution, which produces an IC_{50} value of 4.03 ppm. The smaller IC_{50} value indicates the stronger antioxidant activity of Bidara leaf extract.

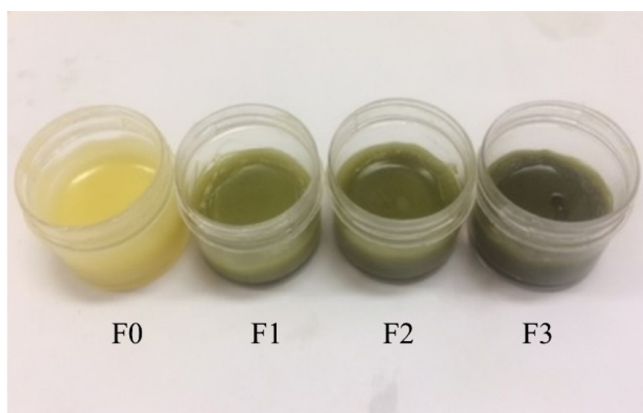
Nevertheless, this result shows a smaller IC_{50} value when compared to the study of Kusriani *et al.* (2015), which has an IC_{50} of 127.87 ppm, and research of Haeria (2016) which has an IC_{50} of 90.9548 ppm. Differences in levels of the secondary metabolites of a compound can be caused by the geographical location of the plant, climatic factors including temperature, air and humidity, essential factors such as light, water and soil nutrients, as well as pest or disease disturbance factors and weeds (Kardono *et al.*, 2003).

3.4 Lip balm preparation result

Lip balm preparations formed different colors (Figure 4). F0 with 0% extract concentration was yellow because it did not contain Bidara leaf extract, F1 with 1% extract concentration had light green preparation, while both F2 (2%) and F3 (3%) had dark green preparation. Bidara leaf extract has a green color due to the presence of chlorophyll compounds (Moraes *et al.*, 2014), so that variations of Bidara leaf extract added to lip balm preparation will produce an intense green color as the increasing of extract added.

Table 3. Antioxidant activity of Bidara Leaf Extract

No	Concentration (ppm)	% Inhibition (SD)	IC50 (ppm)
1	0	0	25.224
2	2.5	28.35 ± 0.0028	
3	5	33.33 ± 0.0014	
4	10	40.23 ± 0.0028	
5	20	47.13 ± 0.0028	
6	40	62.45 ± 0.0014	
7	80	93.49 ± 0.0007	

**Figure 4.** The result of the formulation of Bidara leaf extract lip balm

3.5 Physical quality analysis of lip balm preparations

Analysis of the lip balm formula preparation of Bidara leaf extract includes melting point, pH value, smear test, homogeneity test, stability test, and microbial contamination test to determine the characteristics of each lip balm preparation. Standardization of lip balm preparation products refers to the physical quality requirements of SNI 16-4769-1998.

3.5.1 Melting point

The melting point of lip balm preparations was influenced by the components that make up the lip balm, such as the concentration of hardener, fat, and emollient. This research uses beeswax as a hardener which has a melting point of 62-64 °C, cocoa fat which has a melting point of 35 °C, and emollients, namely sunflower seed oil, and glycerin. A good mixture of wax is not more than 50% because it will produce a lip balm with a

hard texture while the concentration of oil can lower the melting point of the lip balm (Ansel, 2008). Table 4 showed that the four lip balm formulations had identical melting points in the range of 53.5 – 54.5 °C, so therefore the melting point range according to SNI 16-4769-1998.

3.5.2 pH value

Recommended lip balm has an acidity value that is close to the physiological pH value of the lip skin, which is 3.8 - 4.7, under it will cause skin irritation, while above that will cause dry skin (Pundlik *et al.*, 2011). Table 4 was the result of pH examination which shows that the four lip balm preparations have a pH value in the range of 4.43 - 4.53, due to the presence of acidic sunflower seed oil content. The results of the pH test indicate that the preparations made are safe and do not cause irritation to the lips and meet the pH range of skin acceptance according to SNI 16-4769-1998.

3.5.3 Dosage grease test

Good greasing power of lip balm preparations showed with does not provide color (transparent), evenly, and homogeneously when applied. The results of the smear test for the preparation of lip balm preparations of Bidara leaf extract showed that all preparations had good smearing power (Table 4). Although the preparation of Bidara leaf lip balm was green physically, the whole preparation gives a transparent smear test result and indicated that the extract was well dispersed in the lip balm formula so that it does not leave a green color.

3.5.4 Homogeneity

Parameters of homogeneity testing of lip balm preparation were carried out to see the presence or absence of coarse grains. Coarse grains indicated that the lip balm preparation was not homogeneous because it was not dispersed between the components of the lip balm to form a homogeneous composition. The results of the homogeneity examination in table 4 showed that all lip balm preparations of Bidara leaf extract did not show any coarse grains when applied to the transparent glass which means the Bidara leaf lip balm had a homogeneous composition. The formation of good homogeneity will affect the even distribution of lip balm doses at the time of use. Homogeneous lip balm will give good results because the medicinal ingredients are evenly dispersed in the base material so that when applied, the applied dose was evenly distributed and the use of lip balm will be effective in protecting the lips.

3.5.5 Stability

Stability observation was carried out to determine the occurrence of changes in color, scent, and texture or dosage form. The preparations that were stored for 20 days at room temperature did not experience drastic changes in both color and scent. All lip balm preparations began to change in texture on day 15 of storage with the appearance of white spots and increased in number on day 20. This was probably due to the

absence of preservatives that can maintain the stability and durability of the lip balm so that the texture of the lip balm was damaged, meanwhile, the color and scent of the lip balm did not change.

3.5.6 Microbial contamination

The microbial contamination test is one of the tests required by SNI 16-4769-1998. This test was important because microorganism contamination can cause phase separation, sample weight loss, affect shelf life, unpleasant odor, even more, irritability. Cosmetic products contaminated with microorganisms were usually seen from the formation of colored fungal colonies, changes in odor, changes in viscosity that damage the quality of cosmetic preparations (Tranggono and Latifah, 2007). According to Buckle *et al.* (2010), factors that can affect the growth of microorganisms include pH, water activity, temperature, and oxygen content.

The calculation of microbial contamination was carried out using the Total Plate Count method where all colonies that grew in Petri dishes were counted and to meet the statistical requirements, the petri dish selected in the calculation has a colony count of 30 - 300 in several dilutions (Waluyo, 2008). The maximum amount of microbial contamination in mask products according to SNI is 10² colonies/gram. The results of the microbial contamination test on all lip balm preparations did not show the number of microbial colonies in the range of 30-300 colonies so that the calculation of microbial contamination was considered non-existent. Therefore, all lip balm preparations meet the standards based on SNI 16-6070-1999 and are safe to use.

3.6 Preference test result

The preference test is one type of acceptance test to obtain the most preferred lip balm variety preparations by the panelists. The panelists consisted of 21 women and 9 men aged 22 years and over, had knowledge of lip balm, and were willing to become panelists. The level of preference was referred to as a hedonic scale which can be stretched

according to the desired scale range. The hedonic scale can be converted into a numeric scale with quality scores according to the level of preference, with this numerical data, parametric data analysis can be performed (Setyaningsih *et al.*, 2010).

Based on table 4, the results of the general level of preference for lip balm ranged from a score of 2.53 - 4.37, which means that the panelists stated their level of preference from dislike to like. The results of lip balm preparations that have the highest level of preference are F1 with an average score of 4.37 and the lowest level of preference is F3 with a score of 2.53. Both light green (F1) and yellow (F0) preparation of lip balm were more accepted by panelists compared to the dark green preparation left (F2, F3). The effect of smooth texture, light color, and scent that covers the smell of the extract and other compositions, conclude that the F1 preparation was chosen as the best formula on the preference test.

The results of the one-way ANOVA statistical test with a 95% confidence level had a significant value of 0.000 which indicated that there was a significant difference ($P < 0.05$) between the addition of Bidara leaf extract to the lip balm formulation and the panelists' preference level. The entire score generated is considered as a parameter that represents the assessment of the panelists, so the best formulation chosen is lip balm with the addition of 1% extract.

The lip balm product in this study had a melting point, pH, and microbial contamination in the range of SNI 16-4769-1998. Based on the requirements of SNI 16-4769-1998 and the general highest level of panelist preference test, F1 was chosen as the best formula preparation of Bidara leaf extract lip balm which has a melting point of 53.5 °C, pH value 4.48, and homogeneous texture.

Table 4. Characteristic of lip balm preparations of Bidara leaf extract

Parameter	F0	F1	F 2	F3	SNI
Melting point (° C)	54.50	53.50	53.50	53.50	50 – 70
pH	4.53	4.48	4.43	4.45	3.8 – 4.7
Smear test	Colorless, even	Colorless, even	Colorless, even	Colorless, even	
Homogeneity test	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Microbial contamination (colony/gram)	Nothing	Nothing	Nothing	Nothing	Maximal 10 ²
Texture preference test	4.03 ± 0.615	3.97 ± 0.809	3.70 ± 0.794	3.33 ± 0.922	
Color preference test	4.07 ± 0.944	4.00 ± 0.743	3.50 ± 0.900	2.67 ± 0.994	
scent preference test	4.07 ± 0.177	3.90 ± 0.913	3.30 ± 0.988	3.03 ± 1.217	
Homogeneity preference test	4.40 ± 0.563	4.23 ± 0.504	4.00 ± 0.643	4.00 ± 0.371	
General preference	4.23 ± 0.626	4.37 ± 0.556	3.43 ± 0.774	2.53 ± 0.937	

Note: F0 = lipbalm without the addition of Bidara leaf extract; F1 = lipbalm with the addition of 1% Bidara leaf extract; F2 = lipbalm with the addition of 2% Bidara leaf extract; F3 = lipbalm with the addition of 3% Bidara leaf extract

Table 5. Antioxidant activity of F1 lip balm preparation

No	Concentration (ppm)	% Inhibition (SD)	IC50 (ppm)
1	0	0	
2	12.5	1.82 ± 0.0014	
3	25	7.27 ± 0.0028	
4	50	9.55 ± 0.0014	339.234
5	100	18.64 ± 0.0028	
6	200	31.14 ± 0.0007	
7	400	57.50 ± 0.0035	

3.7 Antioxidant activity of lip balm preparation

The addition of natural ingredients in the form of extracts in cosmetics has the potential to maintain the physiological balance of human skin with a low or safe level of toxicity (Chancal and Swarnlata, 2008). Dhuha *et al.* (2020) showed that Bidara leaf extract at doses below 1500 mg/kg BW did not shows a toxic effect and was safe for the kidney function of white rats. So that, the use of ethanol extract for lipbalm preparations is below the safe threshold, including < 7.5% (w/w).

Determination of the antioxidant activity of the best lip balm preparations was carried out to determine how much influence the antioxidant compounds had on the ethanol extract of Bidara leaves which were added to the lip balm formulation. The lip balm formula of F1 has a fairly large IC50 value compared to Bidara leaf extract, by different 314.01 ppm. The heating process in the formulation and addition of lip balm composition is considered to reduce the antioxidant activity of Bidara lip balm caused by damage to the active substance in the extract (Murniyati *et al.*, 2021).

The results showed that the antioxidant activity of the F1 as the best lip balm formula was weak, while the extract was quite strong. This can occur because the ethanol extract content is lower than other lip balm components in line with the results of table 5, which increased the content of Bidara leaf extract, the % inhibition of lip balm preparations is getting better to neutralize free radicals. Other studies showed that processing Bidara leaf extract into cosmetic preparations such as peel-off gel masks and lipsticks also resulted in weak antioxidant activity (Murniyati *et al.*, 2021; Nurhaida *et al.*, 2017). 4.

4. Conclusion

The ethanol extract of Bidara leaves which was identified through GCMS analysis showed that phytol compounds were thought to act as antioxidants. Bidara leaves extract has an IC50 value of 25.224 ppm, which categorized as a fairly strong antioxidant compared to the standard. The result of the preference test showed a significant different at $P < 0.05$, so that the addition of Bidara leaves extract had an effect on the texture, color, aroma, homogeneity and general preference of the panelists. The most preferred lip balm preparation by panelists was formula F1 with the addition of 1% (w/w) ethanol extract of Bidara leaves and has an IC50 value of 339.234 ppm which has weak antioxidant activity. However, all lip balm formulas meet SNI 16-4769-1998 standards with a melting point of 53.5 – 54.5 °C, pH value of 4.43 – 4.53, homogeneous, and negative microbial contamination.

Acknowledgments

The authors would like to thank the Rector of State Islamic University for the research funding project through the LP2M Research Centre for 2021.

References

- Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 2013; 21: 143-152.
- Ansel HC. Introduction to Pharmaceutical Dosage Forms. UI Press, Jakarta, Indonesia. 2008.

- Ashri NH. Activity Test and Identification of Antibacterial Chemical Compounds Ethanol Extract of Bidara Leaves (*Ziziphus Spina-Christi* L.) Against Several Pathogenic Bacteria [Thesis] Alauddin State Islamic University Makassar, Makassar. 2016.
- Aykul S and Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. *Analytical Biochemistry* 2016; 508: 97-103.
- Bintoro A, Ibrahim AM, Situmeang B. Analysis and Identification of Saponin Compounds from Bidara Leaves. *ITEKIMA* 2017; 2(1): 84-94.
- BSN. SNI 16-4769-1998 Lipstick Quality Requirements. National Standardization Agency, Jakarta, Indonesia. 1998.
- BSN. SNI 01-2346-2006 Instructions for Organoleptic and/or Sensory Tests. National Standardization Agency, Jakarta. 2006.
- Buckle KA, Edwards RA, Fleet GH, and Wotton M. Food Science. University of Indonesia Press, Jakarta, Indonesia. 2010.
- Chancal D. and Swarnlata S. Novel Approaches in Herbal Cosmetics. *Journal of Cosmetic Dermatol* -2008; 7: 89-95.
- Dhuha NS, Haeria, Putri HE. Acute Toxicity Ethanol Extract of Bidara Leaves (*Ziziphus Spina-Christi* L.) Against Liver and Kidney Function of White Rats. *Eureka Herba Indonesia* 2020; 2(1): 11-14.
- Draelos ZD, and Thaman LA. Cosmetic Formulation of Skin Care Products. Taylor and Francis Group, New Jersey, USA. 2006.
- Elaloui H, Ghazghazi A, Ennajah S, Manaa W, Guezmir NB, Karray A, Laamouri. Phenolic profile, antioxidant capacity of five *Ziziphus spina-christi* (L.) Willd provenances and their allelopathic effects on *Trigonella foenum-graecum* L. and *Lens culinaris* L. seeds, *Natural Product Research* 2016; DOI: 10.1080/14786419.2016.1226830.
- Goyal AK, Middha SK, Sen A. Evaluation of the DPPH Radical Scavenging Activity, Total Phenols, and Antioxidant Activity in Indian Wild *Bambusa vulgaris* 'Vittata' Methanolic Leaf Extract. *Journal of Natural Pharmaceuticals* 2010; 1(1): 40-45.
- Hadijanah S. Uji Toksisitas Ekstrak Etanol Daun Bidara (*Ziziphus mauritiana*) terhadap Larva Ugang (*Artemia salina* Leach) dengan Metode *Brine Shrimp Lethality Test* (BSLT) (Thesis). Institut Kesehatan Helvetia, Medan. 2018.
- Haeria. Determination of Total Flavonoid Levels and Antioxidant Activity of Ethanol Extract of Bidara Leaf (*Ziziphus spina-christi* L.). *Journal of Pharmaceutical and Medicinal Sciences* 2016; 1(2): 57-61.
- Hendrawati, Aziza, Sumarlin LO, Azizah YN. Formulation, Antioxidant, And Antibacterial Activities Of Peel-Off Gel Mask Enriched With Bidara Leaf (*Ziziphus Spina-Christi* L.) Extract. *International Journal Of GEOMATE* 2020; 18(68): 66 - 72.
- Ikalinus R, Widyastuti SK, Setiasih NLE. Skrining Fitokimia Ekstrak Etanol Kulit Batang Kelor (*Moringa oleifera*). *Indonesia Medicus Veterinus* 2015; 4(1): 71-79.
- Jacobsen PL, Denis PL, Michael AS, Dore E, Barbara DW. The Little Lip Book. Carma Laboratories Inc. Thing, USA. 2011.
- Kardono LBS, Artanti N, Dewiyanti ID, Basuki T, Padmawinata K. Selected Indonesian Medical Plants: Monographs and Description Volume I. Gramedia, Jakarta, Indonesia. 2003.
- Khaleel SMJ, Jaran AS, Haddadin MSY. Evaluation of Total Phenolic Content and Antioxidant Activity of Three Leaf Extract of *Ziziphus spina-christi* (Sedr) Grown in Jordan. *British Journal of Medicine & Medical Research* 2016; 14(6): 1-8.
- Khanahmadi M, Rezazadeh SH, Taran M. In Vitro Antimicrobial and Antioxidant Properties of *Smyrniun Cordifolium* Boiss (*Umberliferae*) Extract. *Asian Journal of Plant Science* 2010; 9(2): 99-103.s
- Kumar PP, Kumaravel S, Latitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African Journal of Biochemistry Research* 2010; 4(7): 191-195.
- Kusriani RH, Nawawi A, Machter E. Determination of Total Phenolic Compound Levels and Antioxidant Activity of Leaf, Fruit and Seed Extract of Bidara (*Ziziphus Spina-Christi* L.). *Proceedings of SNaPP2015 Health* 2015; 1(1): 311-318.

- Kwunsiriwong S. The Study on the Development and Processing Transfer of Lip Balm Products from Virgin Coconut Oil: A Case Study. Official Conference Proceedings of The Asian Conference on Sustainability, Energy & the Environment. Bangkok, Thailand. 2016.
- Mitsui T. New Cosmetic Science. Elsevier, Tokyo. 1997.
- Molynieux P. The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin Journal of Science and Technology 2004; 211–219.
- Moraes J, Oliveira RN, Costa JP, Junior ALG, Sousa DP, Freitas RM, Allegratti SM, Sinto PLS. Phytol, a Diterpene Alcohol from Chlorophyll, as a Drug against Neglected Tropical Disease Schistosomiasis Mansoni. PLOS Neglected Tropical Diseases 2014; 8(1): e2617.
- Murniyati, Subaidah WA, Ananto AD. Formulasi dan Uji Aktivitas Antiradikal Bebas Sediaan Gel Ekstrak Etanol Daun Bidara (*Ziziphus mauritiana* Lamk) Menggunakan Metode DPPH. Jurnal Ilmu Kefarmasian 2021; 2(2): 96-102.
- Nurhaida A, Susilo H, Sari BL. Antioxidant Activity of Lipstick Preparations With Natural Coloring Extract Of Super Red Dragon Fruit (*Hylocereus Costaricensis* L.). Pakuan University, Malang, Indonesia. 2017.
- Pundlik H, Deshpande RR, Bavage NB, Gali V, Bavage SB. Formulation and Evaluation of Herbal Lipstick. International Journal of Innovative Research in Technology; 6(2): 449-453.
- Putri RAZ. Activity Test of Arabic Bidara Leaves (*Ziziphus Spina-Christi* L.) As Anticancer In Colon Cancer Cells (WiDr) Through MTT Method And Identification Of Active Compounds With LC-MS Method. Maulana Malik Ibrahim State Islamic University, Malang, Indonesia. 2017.
- Ratih H, Titta H, Puri RC. Formulation of Cananga Oil (Cananga Oil) Lipbalm as an Emollient. Proceedings of the Synopsis for Research on Natural Medicinal Ingredients (SPBOA) XVI. 2014.
- Saadah H, and Nurhasnawati H. Comparison of Ethanol and Water Solvents in the Preparation of Tiwai (*Eleutherine Americana Merr*) Tuber Extract. Manutung Scientific Journal 2015; 1(2): 149–53.
- Salmiyah S, and Bahrudin A. Phytochemicals and Antioxidants in Tome-Tome Fruit (*Flacourtia Inermis*), 2018; 10(1): 43–50.
- Santos CCMP, Salvadori MS, Mota VG, Costa LM, Almeida AAC, Oliveira GAL, Costa JP, Sousa DP, Freitas RM, Almeida RN. Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. Neuroscience Journal 2013; 949452: 1-9.
- Setyaningsih D, Apriyantono A, Sari MP. Sensory Analysis for the Food and Agro Industry (DS Sard). IPB Press, Bogor, Indonesia. 2010.
- Sulastomo E. Beautiful and Healthy Skin: Knowing and Caring for the Skin. Compass, Jakarta, Indonesia. 2013.
- Tranggono RI, and Latifah F. Handbook of Cosmetic Science. Jakarta: Main Library Publisher; 2007.
- Waluyo L. Basic Techniques and Methods in Microbiology. University of Muhammadiyah Malang Press, Malang, Indonesia. 2008.
- Wibowo DS. Anatomy of the Human Body. Grasindo, Jakarta, Indonesia. 2008.