Formulation of Antioxidant Gel from Black Mulberry Fruit Extract (Morus nigra L.)

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ABSTRACT

Background: Ultraviolet (UV) radiation from the sun that is composed of UVA and UVB can cause premature aging when exposed to the skin. Black mulberry fruit (Morus nigra L.) contains anthocyanins as antioxidants that can be protective against UV exposure. The aim of this research was to produce gel formulation from the extract of black mulberry as an antioxidant and sunscreen.

Materials and Methods: This research started with the maceration process using 96% ethanol solvent. Later, antioxidant activity of the extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and compared with vitamin C. Furthermore, the extracts were formulated into gel with variations of hydroxypropyl methylcellulose, sodium carboxymethyl cellulose, Carbopol 934, and extract concentrations. The products were then physically evaluated, including organoleptic evaluation, homogeneity, pH, viscosity, and dispersion, as well as the hedonic test, irritation test, and antioxidant activity test. Results: The results showed that black mulberry extract had antioxidant activity with the half maximal inhibitory concentration (IC50) value of 146.73 ppm and its antioxidant strength was 39.6 times lower than that of vitamin C. The formulation with best physical evaluation results was given by formula 4 (Carbopol 934, 1.5%) with extract concentration 0.61%. This formula showed antioxidant activity with an IC50 value of 104.659 ppm. Furthermore, on the basis of hedonic test and irritation test, this formula was the most popular and was categorized as a safe topical gel. Conclusion: The gel of black mulberry extract had antioxidant activity and was categorized as a safe topical gel.

KEYWORDS: Antioxidant, black mulberry, gel

INTRODUCTION

The skin is an organ that has direct contact with the environment and protects the body from harmful effects of the environment and environmental damage, such as ultraviolet (UV) rays. However, UV rays cause skin aging faster if there is too much exposure on the facial skin.[1] UV light is divided into two parts, UVA and UVB. UVB rays are absorbed by the epidermis, whereas UVA rays are absorbed by the endodermis. UVB rays cause sunburn that can be prevented by photoprotection or expressed as a sun protection factor values, whereas UVA light absorbed will trigger faster formation of free radicals.[2] Free radicals are highly reactive compounds because they have unpaired free electrons.[3] This can be prevented by using antioxidants.

Some of the antioxidants commonly used in cosmetic preparations are tocopherol, ascorbic acid, and vitamin A.[4] However, the use of synthetic antioxidants can be carcinogenic and toxic in high doses.[5] Natural antioxidants can be used as an alternative, one of...
which is the black mulberry fruit. Compared to other mulberries, black mulberry contains the most phenolic and flavonoid compounds called anthocyanins. These anthocyanin compounds are cyanidin-3-glucoside and cyanidin-3-rutinoside. Previous studies have shown that crude mulberry anthocyanins, quercetin 3-(6-malonylglucoside), and rutin from mulberry leaves (family: Moraceae) are excellent antioxidant agents.

In this research, the extract of black mulberry was preferred to be made into a gel form because it is easy to spread, easy to wear, and comfortable on the skin. The main objective of this research was to determine the best formula of the antioxidant gel from the black mulberry fruit extract.

**Materials and Methods**

**Materials**

**Plant material**

The plant of *Morus nigra* L. was collected from Manda Farm, Maribaya Timur, Cibodas, and West Java and was authenticated (plant authentication no. 590/HB/05/2018) by the Department of Biology, Faculty of Science, Universitas Padjadjaran, Indonesia.

**Chemicals**

The chemical 1,1-diphenyl-2-picrylhydrazyl (DPPH) pro analysis was purchased Sigma Aldrich (St Louis, MO). Vitamin C and methanol pro analysis were obtained from Merck. All other chemicals were of technical grade.

**Methods**

**Preparation of plant extracts**

A total of 10 kg of fresh black mulberry fruit was dried using an oven at 50°C for 3 × 24 h. The dried fruit was extracted using the maceration method with 96% ethanol at room temperature. The ethanol was removed by using a rotary evaporator, IKA company, Germany (IKA RV 10) at 50°C for obtaining a crude extract.

**Screening of phytochemical and chemical content extract test**

The phytochemical screening of black mulberry fruit extract was carried out for the detection of secondary metabolites such as alkaloids, flavonoids, polyphenols, tannins, saponins, quinones, and steroids/terpenoids.

**Antioxidant activity test of black mulberry fruit extract**

The DPPH solution (20 ppm) was prepared using 96% ethanol as a solvent. The sample solution and DPPH (2:3) were mixed and then the mixed operating time was determined. Subsequently, the sample solution was prepared by various concentrations and mixed with DPPH solution (2:3), stored during operating time, and then absorbance was measured at $\lambda_{\text{max}}$ (517 nm). The value of absorbance was calculated for the value of %inhibition by using the equation:

$$\%\text{inhibition} = [1 - \left( \frac{A_{\text{sample}}}{A_{\text{DPPH}}} \right)] \times 100$$

where, %inhibition is the percentage capacity of free radical inhibition, $A_{\text{sample}}$ is the absorbance of the sample, and $A_{\text{DPPH}}$ is the absorbance of DPPH control.

Linear regression curve between %inhibition and sample concentration was made, and the linear equation and IC$_{50}$ values were obtained.

**Formulation of antioxidant gel from black mulberry fruit extract**

Formulation of antioxidant gel from black mulberry fruit extract with gel base variation was made based on the formula in Table 1.

The gelling agent was dissolved in water until the gel base was formed, and then glycerin was added. Black mulberry extract, methyl paraben, and propyl paraben were dissolved into water, added into the gel base mixture, and stirred until a homogenous mass was obtained. Water was added until the gel mass was 100 g. The best gel-based formulas were selected to be incorporated into the extract concentration formulas in

| Table 1: Formulation of antioxidant gel from black mulberry fruit extract with gel base variation |
|--------------------------------------------------|
| Composition | F$_1$ (%) | F$_2$ (%) | F$_3$ (%) | F$_4$ (%) | F$_5$ (%) | F$_6$ (%) | F$_7$ (%) | F$_8$ (%) | F$_9$ (%) |
| Carbopol 934 | 1 | 1,5 | 2 | - | - | - | - | - | - |
| TEA | qs | qs | qs | qs | - | - | - | - | - |
| HPMC | - | - | - | 2 | 3 | 4 | - | - | - |
| Na-CMC | - | - | - | - | - | - | 3 | 3,5 | 4 |
| Glycerin | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Methyl paraben | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Propyl paraben | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Perfume | qs | qs | qs | qs | qs | qs | qs | qs | qs |
| Extract | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ | 1.5xIC$_{100}$ |
| Aquadest ad | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
Table 2, and the physical stability was observed until 28 days.[17]

**Evaluation of antioxidant gel formulas**

Physical stability of gel from black mulberry extract was evaluated, including organoleptic properties, pH, and viscosity for 28 days.[17]

**Spreadability**

One gram of gel was placed between the two glass slides and 125 g weight was added on the glass slide for a minute. The diameter of the gel distribution was taken as a measure of spread ability.[18]

**Antioxidant activity test of black mulberry fruit extract in gel**

Each formula was made in various weight concentrations (0.5, 1, 2, 3, and 4 g). Gel was dissolved in 10mL of 96% ethanol, then synthesized for 20 min, and centrifuged for 10 min at 5000 rpm. Later, the sample solution was mixed with DPPH solution (2:3) and stored during operating time, the absorbance at λ\text{max} was measured and the % inhibition value was obtained. A linear regression curve was made, then linear equation and IC_{50} values were obtained.[3]

**Irritation tests**

The ethical approval for the experimental procedure for the irritation test was obtained from the Health Research Ethics Committee, Faculty of Medicine, University of Padjadjaran (KEPK-FK UNPAD) with number 1291/UN6.C1.3.2/KEPK/PN/2016. The irritation test was performed using the repeated patch test method with the following volunteer criteria:[19]

1. Female sex, healthy, not pregnant
2. Age range between 20 and 35 years
3. Has no history of allergic diseases and is not taking any medications that are anti-inflammatory or cause allergic effects
4. Willing to be a volunteer panelist

The irritation test was performed on the inside of the arm (3 × 3 cm) and then cleaned with NaCl physiological solution. The test was performed for 24 h with four treatments and evaluated for 15 min, 1 h, and 24 h after the preparation was cleared from the arm [Table 3].

**Data analysis**

The data of this work were presented as a mean of sample values ± standard deviation and were analyzed using the one-way analysis of variance (ANOVA) at the level of P < 0.05 to determine if the changes in the applied factors were statistically significant at the level of P < 0.05 and nonsignificant at the level of P > 0.05.

**RESULTS**

The result of phytochemical screening from black mulberry fruit extracts is shown in Table 4, where “+” indicates detected and “-” indicates not detected.

On the basis of phytochemical screening, the extracts were detected to be containing flavonoid compounds, polyphenols, tannins, monoterpenoids, and sesquiterpenoids. The black mulberry fruit is rich in flavonoids and phenols, that is, anthocyanin compounds.[22]

The activity of antioxidant from black mulberry extract and vitamin C is shown in Table 5.

**Formulation of antioxidant gel from black mulberry fruit extract**

On the basis of physical observation, it was found that the best gel-based formulation was formula 2. Carbopol 934 with a concentration of 1.5% was the best and the most optimum concentration for the gel formula. Carbopol gives a cold sensation, leaves no stickiness, and does not cause the separation of phases (syneresis).[23,24]

**Table 2: Formulation of antioxidant gel from black mulberry fruit extract with variation of extract concentration**

| Composition          | F₁ (%) | F₂ (%) | F₃ (%) | F₄ (%) |
|----------------------|--------|--------|--------|--------|
| Gel base             | adjusted to the selected base formula |
| Glycerin             | 10     | 10     | 10     | 10     |
| Methyl paraben       | 0.18   | 0.18   | 0.18   | 0.18   |
| Propyl paraben       | 0.02   | 0.02   | 0.02   | 0.02   |
| Extract              | -      | 1.5xIC\text{100} | 2xIC\text{100} | 3xIC\text{100} |
| Perfume              | qs     | qs     | qs     | qs     |
| Dye                  | qs     | qs     | qs     | qs     |
| Aquadest ad          | 100    | 100    | 100    | 100    |

**Table 3: Response of irritation**

| Respon Value | Level of Irritation |
|--------------|---------------------|
| 0 – 0.4      | meaningless         |
| 0.5 – 1.9    | low irritation      |
| 2.0 – 4.9    | medium irritation   |
| 5.0 – 8.0    | severe irritation   |

**Table 4: Phytochemical screening of black mulberry fruit extracts**

| Compound                          | Results |
|-----------------------------------|---------|
| Alkaloids                         | -       |
| Flavonoids                        | +       |
| Saponins                          | -       |
| Polyphenols                       | +       |
| Monoterpenes and sesquiterpenes   | +       |
| Tanins                           | +       |
| Quinons                          | -       |
| Steroids/triterpenoids            | -       |

Where (+) was detected and (-) was not detected.
Organoleptic properties and homogeneity

On the basis of observations, it was found that the extract only affected the organoleptic properties in terms of color. The high concentration of extract can change the color of gel. The odor, consistency, and homogeneity did not show significant change.

**pH**

The pH measurement result of gel is shown in Figure 1. A good pH for the formula is the one corresponding to the pH range of the skin, that is, 4.5–6.[18]

During storage time, the most stable formula was formula 4. On the basis of results of statistical analysis, the $P$ value was found to be more than 0.05; therefore, $H_0$ was accepted and $H_a$ was rejected, which means that no significant difference was observed between pH in each formula during storage time.

**Viscosity**

Viscosity affects long storage period. The increased viscosity will affect the dispersion and release of the active substance in the formula. During storage time, the formulas tend to increase in viscosity. The optimum viscosity will retain the active substance to be dispersed and maintain uniformity of concentration on the base [Figure 2].[25]

The results of statistical analysis showed that the $P$ value was more than 0.05; therefore, $H_0$ was accepted and $H_a$ was rejected, which means that no significant difference was observed between the viscosity in each formula and storage time.

| Table 5: IC$_{50}$ value of antioxidant from black mulberry extract and vitamin C |
|-------------------------------|------------------|
| Samples                      | IC$_{50}$ (ppm)  |
| Black mulberry extract        | 146.73 ± 12      |
| Vitamin C                     | 3.70 ± 0.2       |

**Spreadability**

The dispersion in the formula that contains the active compound, such as the antioxidant gel, plays a vital role as the absorption medium and the release of the active substance. A good scattering requirement for the formulas is in the range of 5–7 cm [Figure 3].[16]

Spreadability is inversely proportional to viscosity. Increased viscosity will cause the decrease of dispersion.[16] On the basis of analysis using one-way ANOVA, the significant value was 0.115 ($P > 0.05$); therefore, $H_0$ was accepted, which means that no difference was observed in power output spread at various storage times. This shows that there is no effect of spreadability on the storage time.

Antioxidant activity test of black mulberry fruit extract in gel

Tests were performed as much as three times to observe the antioxidant activity during the storage period. On the basis of observations, formula 4 provided the most powerful antioxidant activity ability with an IC$_{50}$ value of 104.65 ppm [Table 6]. The ability of antioxidant activity with IC$_{50}$ value of 100–250 ppm on each formula belongs to medium strength category.[26]

Statistical analysis using one-way ANOVA showed the significant value as 0.765 ($P > 0.05$); therefore, $H_0$ was accepted, which means that no difference in antioxidant activity was observed at various storage times. This shows that there is no effect of antioxidant activity on the storage time.

Irritation tests

The ethical approval for the experimental procedure for the irritation test was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran (KEPK-FK UNPAD). The test was performed by the repeated patch test method. This method was chosen because the application of gel...
was in the multiple dose preparation. The formulation of gel from black mulberry extract did not give the irritating effect for all administration. These results indicate that antioxidant gel preparations of black mulberry fruit extracts can be safely used as a topical formulation.

**DISCUSSION**

**Preparation of plant extracts**

The drying process at 50°C was carried out to prevent the growth of fungus because the water content of black mulberries was very high (85.5%) with good pH for fungus growth (pH 5) and to keep the stability of anthocyanins. The anthocyanins were stable at low temperature and the purple color faded at temperature greater than 80°C. However, this drying process still degraded the anthocyanins. In total, 846.66 g of simplicia were obtained.

The extraction was performed using the maceration method to keep the stability of anthocyanin. Ethanol (96%) is the solvent that can attract the highest concentration of anthocyanin compared to other solvents and is often used to attract antioxidant compounds in the fruit. Maceration was carried out 2 × 24h because of the color change of macerate on the 2nd day. There was an increase in the yellowish color in the extract due to increased solvents and the bathochromic effect.

The thickening of the extract was performed using a rotary evaporator because anthocyanin compound is thermolabile. However, in this process, anthocyanin degradation was still present, and it gradually removed the purple color. Total anthocyanin may be reduced by 50% when exposed to light or temperature at 70°C after the first 10h. The yield obtained was 44.417%. The extract was stored in the refrigerator to reduce the rate of the anthocyanin color change.

**Antioxidant activity test of black mulberry fruit extract**

The DPPH method was chosen because the DPPH has high solubility in 96% ethanol. This corresponds to the extract of black mulberry fruit, which has the highest antioxidant activity in 96% ethanol solvent. The IC₅₀ value of vitamin 46 C solution was 3.70 ppm. According to Jun et al., IC₅₀ <50 ppm is classified as an extremely powerful antioxidant. Vitamin C has four hydroxyl groups, therefore, the antioxidant activity of vitamin C is powerful enough to make DPPH a stable radical. Although the IC₅₀ value of the extract solution of black mulberry fruit is 146.731 ppm, according to Jun et al., IC₅₀ value between 100 and 250 ppm is classified as a medium antioxidant. According to McGhie et al., the functional group examination using nuclear magnetic resonance spectrophotometry showed that the compound has many hydrogen atoms, which allow the compound to easily capture single electrons in free radicals. The extract solution of black mulberry fruit has an antioxidant activity 39.6 times lower than that of vitamin C.

**Spreadability**

The formulas were stored in gel containers at room temperature, whereas antioxidant compounds, that is, anthocyanins were stable in dark places and cold temperatures (refrigerator temperature). Factors that affect the stability of anthocyanins during storage were pH, temperature, light, and oxygen levels.
CONCLUSION

Black mulberry fruit extract (M. nigra L.) was known to provide antioxidant activity with an IC₅₀ value of 146.73 ppm and antioxidant power 39.6 times lower than that of vitamin C. The best antioxidant gel was the gel containing 1.5% of Carbopol 934 and concentration than that of vitamin C. The best antioxidant gel was the 146.73 ppm and antioxidant power 39.6 times lower to provide antioxidant activity with an IC₅₀ value of 104.65 ppm. Black mulberry fruit extract (M. nigra L.) gave antioxidant activity with an IC₅₀ value of 104.65 ppm.

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Conflicts of interest

There are no conflicts of interest.

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