Antibacterial and Antioxidant Activity of Black Mulberry (Morus nigra L.) Extract for Acne Treatment

Arif Budiman1*, Diah Lia Aulifa2, Arif Satria Wira Kusuma1, Astri Sulastri1

ABSTRACT

Introduction: Black mulberry is rich in phenols and is hence usable in the treatment of acne. It also contains anthocyanin, a well-known antioxidant. This study aimed to examine the antibacterial and antioxidant properties of black mulberry (Morus nigra L.) extract as a potential raw material for use in beauty care products. Method: Fruit extract was obtained using maceration method with 96% ethanol. The antibacterial activity of the extract was determined by disc diffusion method, while the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by microdilution method. Furthermore, the antioxidant activity of the extract was tested by DPPH method. Result: The results showed a 2.5% MIC against S. epidermidis and P. acnes, confirming the antibacterial activity of black mulberry extract. The MBC values for the respective bacterium were 2.5% and 5%. Regarding antioxidant activity, the IC50 value of black mulberry extract was 146.731 mg/mL, suggesting its medium potential. Conclusion: Morus nigra extract has antibacterial activity against S. epidermidis and P. acnes, and has medium potential as antioxidant.

Key words: Morus nigra extract, Antioxidant, Anti acne, Staphylococcus epidermidis, Propionibacterium acnes.

INTRODUCTION

Our skin protects the body from environmental influences such as ultraviolet rays of the sun and numerous microbes. A notable influence of the environment on our skin is premature aging and acne. Premature aging is usually caused by frequent exposure to ultraviolet rays. Ultraviolet rays can cause skin burn and trigger the formation of free radicals more quickly. Acne is an inflammatory disease of the skin that often occurs in adolescence. One of the factors that cause acne on face is due to the activity of bacteria. Propionibacterium acnes and Staphylococcus epidermidis, the two normal skin flora, are often isolated from lesions of acne.

Black mulberry (Morus nigra) contains the highest total phenolic compounds compared to the species of another genus Morus and has antibacterial and anti oxidant activity. 2-arylbenzofuran (Moracin M) has known antibacterial activity against Streptococcus faecalis (MBC 500 µg/mL), and oxresveratrol stilbenoid against Staphylococcus aureus (MBC 125 µg/mL). Mulberry fruits are a rich source of flavonoids and anthocyanin compounds as antioxidant.

MATERIALS AND METHOD

Materials

The materials used in this study consisted of Black Mulberry fruit obtained from Plantations in Cibodas, Maribaya-Lembang, 1.1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), glycerin (Brataco Chemical), hydroxypropyl methylcellulose (HPMC) (Brataco Chemical), Mueller Hinton Agar (Merck), Mueller Hinton Broth (Merck), saline (Otusu-NS), ethanol 96% (CV Sarana Abdi Bakti), Dimethyl Sulfoxide (DMSO) (Merck), P. acnes and S. epidermidis bacteria from the Laboratory of Microbiology, Faculty of Pharmacy University of Padjadjaran.

Extraction

Black mulberry was dried to a final weight of 10 kg in an oven at a temperature of 50°C twice for 24 hours at room temperature. A viscous extract was obtained using a rotatory evaporator under vacuum at 50°C.

Phytochemical Screening

Phytochemical screening of ethanol extract Black Mulberry fruit (Morus nigra) was tested for the presence of alkaloids, steroids/ triterpenoids, saponins,
polyphenols, tannins, flavonoids, quinones, monoterpenoid, and sesqui-terpenoids.

**Antibacterial activity**
The antibacterial activity was tested by disc diffusion method. The growth medium used was Mueller Hinton Agar. Firstly, the extract was dissolved in 0.01% DMSO to obtain four concentrations: 10%, 20%, 40%, and 80%. A 6 mm diameter paper disc was soaked in 5 mL of the extract for 15 minutes and then dried in a laminar flow cabinet for 2 hours. The paper discs were then placed on the surface of the media containing inoculated bacteria. The Petri dishes were incubated at 37°C for 18 hours.

**Determination of MIC and MBC**
MIC and MBC were determined by micro-dilution method using a microplate. 100 mL MHB media added into 100 μL of extract. Furthermore, each well was added 10 mL of the bacterial suspension was adjusted to 0.5 McFarland standards. Furthermore, the microplate was covered with a plastic wrap and then incubated at 37°C for 18 hours.

**Antioxidant activity**
The antioxidant activity of the extract was measured based on its scavenging activity of 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals according to the method described previously with slight modifications. One milliliter of 0.1 mM DPPH solution in methanol was mixed with 1 ml of plant extract of varying concentrations (100, 120, 140, 160, and 180 μg/mL).

**Sample solution**
The DPPH solution (2:3) was allowed to stand for the operating time to read absorbance at the maximum wavelength. A mix of 1 ml methanol and 1 ml DPPH solution was used as the control. Corresponding blank samples were prepared and L-ascorbic acid (1–100 μg/ml) was used as a reference standard. The reaction was carried out in triplicate, and the decrease in absorbance was measured at 517 nm after 30 minutes in the dark using a UV-Vis spectrophotometer. Absorbance values were expressed as a percentage of its inhibition (IC₅₀) using the equation:

\[
\% \text{ Inhibition} = \frac{1 - (A_{\text{sample}}/A_{\text{DPPH}})}{1} \times 100
\]

Where
- % Inhibition = percentage of free radical inhibition capacity
- \(A_{\text{sample}}\) = absorbance of sample
- \(A_{\text{DPPH}}\) = absorbance of DPPH control

IC₅₀ value of the sample was obtained by entering a value of 50 into the equation of each sample.

**RESULTS AND DISCUSSION**

**Plant determination**
Based on the records available at the Department of Biological Science of Universitas Padjadjaran, the plants used in the research were identified as *Morus nigra* L.

**The drying and Extraction**
The purplish black color of black mulberry is due to anthocyanin compounds present in it. These compounds have been found to remain stable in the acidic atmosphere (pH <5) at 50°C, so the drying temperature should not be more than 50°C. The maceration process was limited to 2×24 hours to obtain maserat of consistent color from black mulberry. According to previous study, the highest antioxidant compound of *Morus nigra* extract can be found in a polar solvent. Extraction process using ethanol 96% can attract secondary compounds from botanicals.

Ethanol often used to attract the antioxidant compounds in fruit extracts such as anthocyanin.

**Phytochemicals screening**
The results of phytochemical screening showed can be seen in Table 1. Using phytochemical screening, we detected flavonoids and phenolics in black mulberry. The presence of anthocyanin was also identified, which gives the extract of black mulberry the characteristic violet color.

**Table 1: The Results of Phytochemical Screening of Morus nigra Extract**

<table>
<thead>
<tr>
<th>Secondary metabolic</th>
<th>Black Mulberry Fruit (Morus nigra) Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Monoterpenoids and sesquiterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroid and triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Quinone</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = presence, - = absence

**Table 2: Antibacterial activity**

<table>
<thead>
<tr>
<th>Extract Concentration (% b/v)</th>
<th>Inhibition Zone Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>10</td>
<td>6.50 ± 2.19</td>
</tr>
<tr>
<td>20</td>
<td>12.28 ± 2.82</td>
</tr>
<tr>
<td>40</td>
<td>15.33 ± 0.61</td>
</tr>
<tr>
<td>80</td>
<td>19.46 ± 1.86</td>
</tr>
<tr>
<td>Solvent control DMSO 0.01%</td>
<td>0</td>
</tr>
</tbody>
</table>

**MIC and MBC of Extracts Black Mulberry Fruit (Morus nigra)**

**Table 3: The Results of MIC and MBC of Morus nigra Ethanol Extract**

<table>
<thead>
<tr>
<th>Extract Concentration (% b/v)</th>
<th>The Growth of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. acnes</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>+</td>
</tr>
<tr>
<td>1.25</td>
<td>+</td>
</tr>
<tr>
<td>0.625</td>
<td>+</td>
</tr>
<tr>
<td>0.3125</td>
<td>+</td>
</tr>
<tr>
<td>0.156</td>
<td>+</td>
</tr>
<tr>
<td>0.078</td>
<td>+</td>
</tr>
<tr>
<td>Media Control MHB</td>
<td>-</td>
</tr>
<tr>
<td>Bacteria Control</td>
<td>+</td>
</tr>
<tr>
<td>Extract Control</td>
<td>-</td>
</tr>
</tbody>
</table>
**CONCLUSION**

Black mulberry fruit extracts have antibacterial activity with MIC value of by 2.5% against the bacteria S. epidermis and P. acnes. Black mulberry fruit extracts have antibacterial activity with MBC value for each bacterium was 2.5% and 5%. Black mulberry fruit extracts have medium potential antioxidant activity with IC50 value is 146.731 mg/mL.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest, financial, or otherwise regarding the publication of this paper.

**ABBREVIATIONS USED**

MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentration; DPPH: 1.1-diphenyl-2-picrylhydrazyl; MHA: Mueller Hinton Agar; MHB: Mueller Hinton Broth; DMSO: Dimethyl sulfoxide.

**REFERENCES**


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**Figure 1:** Relationship between concentrations of vitamin C and percentage inhibition of DPPH

**Figure 2:** Relationship between concentrations of black mulberry extract and percentage of inhibition of DPPH

P. acnes was in the range of 2.5–5%. The MBC value of black mulberry against S. epidermis was 2.5%, while that against P. acnes was 5%. We can conclude from the above findings that the ethanol extract of black mulberry has excellent antibacterial activity against S. epidermis and P. acnes at a concentration of 2.5%.

**Antioxidant activity**

The antioxidant activity test based on DPPH method is one of the most common methods for preliminary testing of plant extracts. This method is most suitable for polar compounds such as anthocyanins because the DPPH crystals can easily dissolve in a commonly available solvent and give maximum absorbance.

According to previous study, the antioxidant activity using ethanol 96% has the value of % inhibition is the highest compared with other solvents. This method can be applied to the antioxidant compound that is hydrophilic and lipophilic.

Relationship between concentrations of vitamin C and percentage inhibition of DPPH can be seen in Figure 1. These graphs show that the IC50 of a solution of vitamin C is 3.7057. According previous study, an antioxidant is considered to be very powerful if its IC50 <50 mg/mL. The hydrogen atom in the hydroxyl group binds to free radicals, thus increasing the stability of free radicals. Vitamin C has four hydroxyl groups and so its antioxidant activity is considered to be very powerful. These hydroxyl groups can give electrons to free radicals.

Relationship between concentrations of black mulberry extract and percentage of inhibition of DPPH can be seen in Figure 2.
Black mulberry fruit extracts have antibacterial activity with MIC value of by 2.5% against the bacteria S. epidermidis and P. acnes
Black mulberry fruit extracts have antibacterial activity with MBC value for each bacterium was 2.5% and 5%.
Black mulberry fruit extracts has medium potential antioxidant activity with IC50 value is 146.731 mg/mL.