

## RESEARCH ARTICLE

# Peel-off Mask Formulation From Black Mulberries (*Morus Nigra* L.) Leaves Extract as a Tyrosinase Inhibitor

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## ABSTRACT

Hyperpigmentation is a skin pigmentation disorder caused by increasing melanin production. Tyrosinase enzyme activity plays a role in the melanin pigment synthesis in the skin using tyrosine as a substrate. An inhibitor of tyrosinase enzyme activity is the most prominent method for preventing hyperpigmentation. *Morus nigra* L. (MN) leaves with high flavonoids and polyphenols are the potential material to prevent hyperpigmentation by inhibiting tyrosinase enzyme activity. The aim of this study was to formulate and evaluate a peel-off mask gel of MN leaves extract (*Morus nigra* L.), which can inhibit tyrosinase enzyme activity. The MN leaves were extracted by a maceration method using ethanol (70%) as a solvent. The inhibitor of tyrosinase enzyme activity from MN leaves extract was determined by in-vitro study. The extract was formulated into a base of a peel-off mask gel containing variations in the concentration of polyvinyl alcohol (10, 12.5, and 15%) and gelling agents. The peel-off mask gel of MN leave extract was evaluated, including organoleptic, homogeneity, pH, viscosity, spreadability, and drying time. Irritation tests were performed, and the test of preference of the formulation was also assessed. The result showed that MN leaves extract has an enzyme inhibitor activity of tyrosinase with IC<sub>50</sub> of 511.91 µg/mL. The formulation containing 1.5% of MN leaves extract, 15% of polyvinyl alcohol, and 0.5% of carbomer showed the best physical stability results and safe for topical preparations. It can be concluded that the peel-off mask gel prepared from MN leaves extract is potential for preventing hyperpigmentation as well as safe to be used as a topical preparation.

**Keywords:** *Morus nigra* L. leaves extract, Peel-off mask gel, Tyrosinase.

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## INTRODUCTION

Hyperpigmentation is a skin disorder due to exposure to strong sunlight and can cause darker skin or dark spots. Many people who suffered from dark spots due to hyperpigmentation have a psychological disturbance. Hyperpigmentation in the skin is caused by increasing melanin production.<sup>1,2</sup> Although melanin has the main function of protection from UV radiation. The production of excessive melanin syntheses such as melasma or ages spot can be a severe aesthetic problem.<sup>3</sup>

The pathway for the formation of melanin is called melanogenesis, a process aided by the enzyme tyrosinase.<sup>4</sup> The synthesis of melanin starts with the conversion of l-tyrosine to l-3, 4-dihydroxyphenylalanine (L-DOPA). Tyrosinase enzyme plays a role in the conversion of tyrosine to dopaquinone via L-DOPA.<sup>5</sup> To prevent hyperpigmentation, Melanin biosynthesis can be inhibited by inhibiting tyrosinase activity.<sup>3</sup>

Many topical products are available to reduce the production and distribution of melanin. Because tyrosinase is the key regulator in the production of melanin, several inhibitors of tyrosinase were reported from both synthetic and natural sources. However, only a few materials are being used in topical products due to the efficacy, specificity, and safety concerns. One of the natural sources that can be used as a tyrosinase inhibitor is black mulberry leaves (*Morus nigra* L.). Black mulberry plants are potential as skin depigmentation materials due to an inhibitor of tyrosinase.<sup>5</sup> *Morus nigra* L. (MN) leaf extract has activity as a tyrosinase inhibitor with the inhibition rate reaching 90% at 15.625 mg/mL and had IC<sub>50</sub> value of about 5.00-8.49 mg/mL. Containing chlorogenic acid, rutin, and isoquercetin, MN leaves can inhibit the biosynthesis of melanin, which is closely related to hyperpigmentation.<sup>6</sup>

In this study, we formulated and evaluated a peel-off gel mask of MN leaves extract (*Morus nigra* L.), which can inhibit

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tyrosinase enzyme activity. The peel-off face mask gel is one of the popular, topical dosage form used to enhance the quality of the facial skin.<sup>7,8</sup> The skin face mask has the advantage of being easily peeled off or removed as an elastic membrane.<sup>9</sup> Peel-off gel mask can moisturize the skin and enhances the effects of the main compound in the epithelium due to the occlusivity of the polymer layer formed.<sup>10,11</sup>

## MATERIALS AND METHODS

### Materials

#### Plant material

The plant of MN was collected from Maribaya Timur, Cibodas, and West Java and was authenticated by the Department of Biology, Faculty of Science, Universitas Padjadjaran, Indonesia.

#### Chemicals

The tyrosinase enzyme and Kojic acid were purchased from Sigma Aldrich. All other chemicals were of technical grade.

#### Extraction

The leaves of MN were dried using an oven at 35°C-40°C. The dried leaves were extracted using a maceration method using ethanol 70 % for 3 x 24 hours at room temperature. To obtain a crude extract, the solvent was removed by a rotary evaporator, IKA company, Germany (IKA RV 10) at 60°C.<sup>12,13</sup>

#### Phytochemical Screening Extract

The phytochemical screening of *Morus nigra* leaves extract were conducted for the detection of secondary metabolites such as alkaloids, flavonoids, polyphenols, saponins, quinones, steroids/triterpenoids, tannins, monoterpenes and sesquiterpenes.<sup>14</sup>

#### In Vitro Testing Of Tyrosinase Extract Inhibitory Activity

Extracts were dissolved in dimethyl sulfoxide (DMSO) to give of 10,000 mg/mL. A solution of extract was diluted using 50 mM phosphate buffer (pH 6.5) to obtain solution concentrations

of the extract with a range of 0.5-2000 mg/mL. A total of 70 mL of the extract solution of each dilution was added to 30 mL of the enzyme tyrosinase (Sigma, 333 units/mL in phosphate buffer). Plates were incubated for 5 min at room temperature, then added with 110 mL of substrate 2 mM L-tyrosine and back incubated for 30 min at room temperature. The solution in each well was measured using a microwell plate reader at a wavelength of 510 nm to determine the percent inhibition and the value of 50% inhibitory concentration (IC<sub>50</sub>). The percentage of tyrosinase activity inhibition was calculated as inhibition (%) =  $[(A-B)/A] \times 100$ , where *A* represents the absorbance of the control, and *B* represents the absorbance of the test samples. Kojic acid was used as a positive control.<sup>3</sup>

#### Peel-off Mask Gel Formulation From *Morus Nigra* Leaves Extract

The formulation of peel-off mask gel from MN leaves extract was made according to the formula presented in Table 1. Polyvinyl Alcohol (PVA) was mixed with distilled water (80°C) with constant stirring using a mechanical stirrer, and then *hydroxypropyl methylcellulose* (HPMC) or carbomer was dispersed into the PVA solution. Extract and preservatives were dissolved in glycerol before being added to the PVA/HPMC solution. The mixtures were stirred until homogenous. Physical stability of peel-off mask gel formulation from the MN leaves extract was evaluated through organoleptic, pH, viscosity, dispersion, and the drying time until 21 days.<sup>9,14,15</sup>

#### Drying Time

The sample of the gel was placed and spread over the glass plates 7.0 cm × 2.5 cm. The glass plate was placed in the oven at 37°C for 1-hour. The gel was evaluated until it was completely dry and easily peeled off as a film layer. The drying time of gel was observed until 21 days.<sup>8</sup>

#### Coverage Test

The sample of the gel (0.5 g) was placed on a 20 cm × 20 cm glass and covered with another glass of the same size. Weights

**Table 1:** The formulation of Peel-off Gel Mask Black Mulberry Leaf Extract (*Morus nigra L.*)

Material	Formula (%)					
	I	II	III	IV	V	VI
Mulberry leaf extract	1.5	1.5	1.5	1.5	1.5	1.5
PVA	10	12.5	15	10	12.5	15
HPMC	-	-	-	2	2	2
Carbomer 940	0.5	0.5	0.5	-	-	-
Triethanolamine	qs	qs	qs	-	-	-
Glycerin	10	10	10	10	10	10
Methylparaben	0.18	0.18	0.18	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02
Berrys Essence	qs	qs	qs	qs	qs	qs
Distilled water ad (mL)	100	100	100	100	100	100

**Table 2:** Classification of response categories based on irritation index<sup>16</sup>

Category	Irritation Index
No irritation (no erythema)	0 - 0.4
Slight irritation (slight erythema)	0.5 - 1.9
Moderate irritation (moderate erythema)	2.0 - 4.9
Severe irritation (moderate to severe erythema)	5.0 - 8.0

of 125 g were placed on top of the glass, and the diameter of the gel was measured after 1 min. The coverage test was observed until 21 days.<sup>9</sup>

### Irritation Test Preparations Gel Mask Peel off

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). Irritation test was performed using a repeated patch test method on 12 volunteers with the following criteria: (a) The inclusion criteria as follows: (i) Women aged 18-24 years (ii) Having a normal and healthy skin (iii) provided informed consent for participation. (B) Exclusion criteria as follows: (i) Having a previous skin allergy history, (ii) Currently undergoing topical treatment or using certain products for skin treatment, (iii) Having skin disease, (iv) Presence of any lesions marks such as tattoos, or scars on the test area, and (v) participants of other studies. The part of the arm was cleaned with 70% alcohol, then 1 g of the gel was applied on the cleaned area (3 cm x 3 cm). Aqua distilled was used as a positive control and SDS 0.5% as a negative control. The irritation test was carried out at 15 min, 1 h, and 24 h after the gel application was cleaned.<sup>16,17,18,19</sup>

### Statistical Analysis

The data of experiments, including physical evaluation, irritation test, and product preference test that are presented as a mean of samples  $\pm$  standard deviation (SD), were statistically analyzed using the one-way analysis of variance (ANOVA) method. If the data were not normally distributed, then the Kruskal–Wallis analysis method was used.<sup>20</sup>

## RESULTS AND DISCUSSION

Base on the determination in the Department of Biology, Faculty of Science, Universitas Padjadjaran, the species of black mulberry leaves used in this research is *Morus nigra L.* The maceration method was used in this research to protect the compounds; especially, the compounds were responsible as inhibitors of tyrosinase enzyme contained in MN leaves from thermal decomposition.<sup>14</sup> The solvent used in the maceration process was 70% ethanol because it is a universal solvent that can extract polar and non-polar compounds and it is safe for topical dosage form.<sup>21</sup> The percent recovery of MN leaves extract after evaporated was 21.83%.

The aims of the phytochemical screening test were to determine the presence of secondary metabolites in MN leaves. The result of phytochemical screening of MN leaves extract showed a positive result on flavonoids, polyphenols, tannins, steroids and triterpenoids, and saponins. These results can support the presence of the tyrosinase inhibitor compound in black mulberry leaves such as flavonoids and polyphenols.<sup>6</sup>

To determine the inhibitory potential of the MN leaves extract on the enzyme tyrosinase, the MN leaves extracts were tested and compared with kojic acid as a positive control. To observe the inhibition profile of the MN leaves extracts on tyrosinase activity, the  $IC_{50}$  values were evaluated by the dose-response curves through serial dilution of the MN leaves

extracts and kojic acid at concentrations of 2000  $\mu\text{g/mL}$  to 50  $\mu\text{g/mL}$ .<sup>6</sup> The calculated  $IC_{50}$  values of MN leaves extract was 511.91  $\mu\text{g/mL} \pm 10.4$ , whereas the  $IC_{50}$  of kojic acid obtained was 47.19  $\mu\text{g/mL} \pm 2.5$ . This result indicated that MN leaves extract had lower activity than kojic acid but still has a potential material as a tyrosinase inhibitor. In statistical analysis, the  $IC_{50}$  of MN leaves extracts showed a significant difference ( $p < 0.05$ ) compared to  $IC_{50}$  of kojic acid.

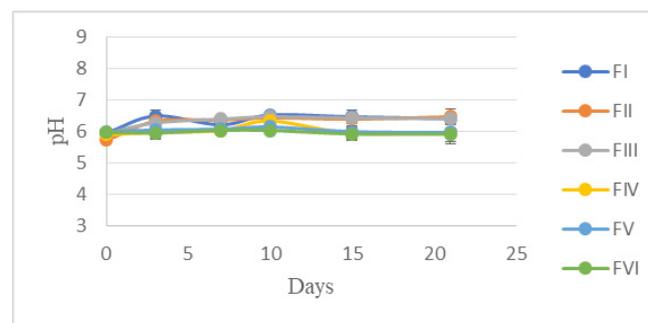
Even though the  $IC_{50}$  result of MN extract has lower compare to the previous report, the extract of MN leaves still showed activity as a tyrosinase inhibitor. This result indicated that the MN leaves extract still contains phenolic compounds such as flavonoids, anthocyanins, and phenolic acids, which have remarkable potential for tyrosinase inhibition.<sup>22-25</sup> This data also was supported by the phytochemical screening test of MN leaves extracts.

The peel-off gel of the MN leaves extract has advantages such as easy to use, has a longer contact time, has high water content, and can reduce the risk of inflammation.<sup>14</sup>

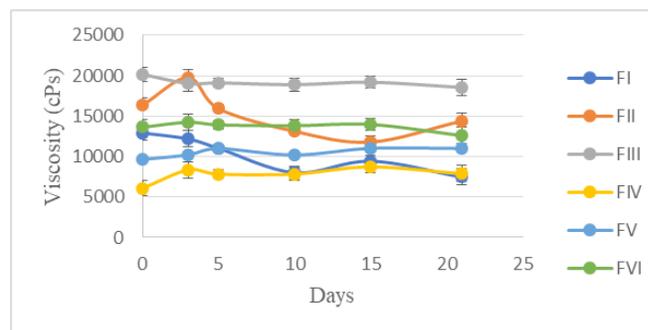
The pH of the peel-off gel of MN leaves extract can be seen in Figure 1.

Base on pH measurements, the pH of peel-off mask of MN leaves extract still remained within the acceptable pH for topical preparations. The acceptable pH of topical preparations is in the range 4.5–6.5 to avoid irritation of the skin.<sup>9</sup> Based on the results of statistical analysis by ANOVA, the value of significance was 0.020 ( $P < 0.05$ ), which indicates that there was an effect of storage on the pH of peel-off mask.

The viscosity of peel-off gel of MN leaves extract can be seen in Figure 2.



**Figure 1:** pH measurement result of peel-off mask of black mulberry leaves extract (The results were calculated as mean  $\pm$  SD; n = 3)



**Figure 2:** Viscosity of peel-off mask gel of black mulberry leaves extract (The results were calculated as mean  $\pm$  SD; n=3)

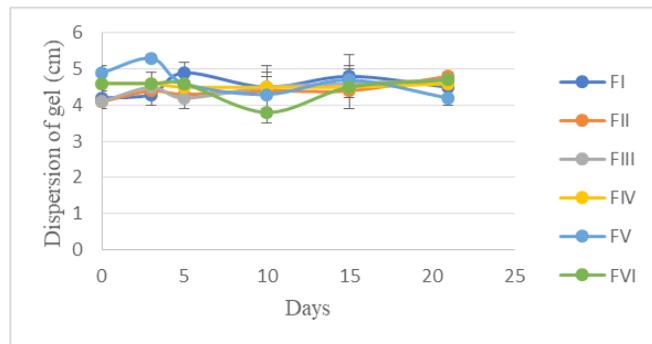
The peel-off gel containing carbomers has a higher viscosity compared to a formula containing HPMC. The hydroxyl (-OH) groups in the carbomer can affect higher viscosity in the peel-off mask gel of MN leaves extract.<sup>26</sup> Based on the results of statistical analysis by ANOVA, the value of significance was 0.010 ( $p < 0.05$ ), which indicated that there was an effect of storage time on the viscosity of the peel-off gel. Even though the storage time can affect the viscosity of peel-off mask gel, the viscosity of peel-off remained within the acceptable viscosity for topical preparations.

The result of dispersion test of peel-off gel can be seen in Figure 3.

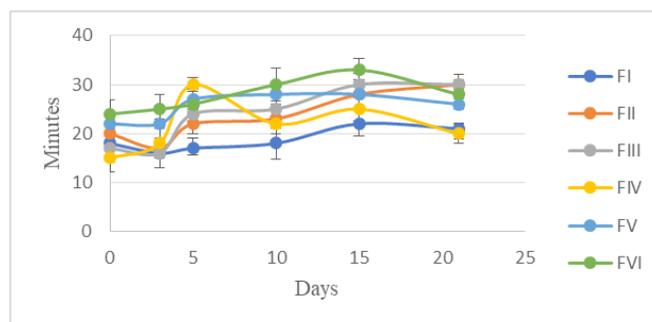
The dispersion test was carried out to evaluate the ability of the peel-off gel spreads during the storage period. The good dispersion ability of peel-off mask gel is 5-7 cm.<sup>9</sup> Increasing the size of the gelling agent can affect the adsorption of solvent and can increase the resistance of peel-off mask gel to flow and spread. Based on the results of statistical analysis by ANOVA, the value of significance was 0.581 ( $p < 0.05$ ), which indicated that there was not an effect of storage time on the dispersion ability of peel-off gel.

The drying time measurement of peel-off gel of MN leaves extract can be seen in Figure 4.

The aims of drying time measurement were to determine the drying time of peel-off gel during storage and compared to the ideal characteristics of the drying time of peel-off gel. The ideal characteristic of the drying time of peel-off gel is 15-30 minutes.<sup>26</sup> Based on the results of statistical analysis by ANOVA, the value of significance was 0.025 ( $p < 0.05$ ), which indicated that there was an effect of storage time on the drying



**Figure 3:** The Dispersion test result of peel-off mask gel of black mulberry leaves extract (The results were calculated as mean  $\pm$  SD; n = 3)



**Figure 4:** The drying time result of peel-off mask gel of black mulberry leaves extract (The results were calculated as mean  $\pm$  SD; n = 3)

time of peel-off gel. However, the result of drying time of peel-off gel after storage remained within the ideal characteristic of peel-off gel for topical preparations.

The formula of peel-off gel containing 15% of PVA and 0.5% of carbomer (FIII) was selected in irritation test due to a better stability base for physical stability and the most convenience formula compared to another formula. The SDS 0.5 % as positive control showed a low irritation while FIII, formula without extract and aqua distilled as negative control did not show the irritation belonging to a category of no irritation. From these results it can be concluded that the preparation of MN leaf extract (*Morus nigra L.*) extract peel-off gel mask does not cause irritation.

## CONCLUSION

Black mulberry leaves extract had an enzyme inhibitor activity of tyrosinase with IC<sub>50</sub> of 511.91 ppm. The formulation containing 1.5% of black mulberry leaves extract, 15 % of polyvinyl alcohol 15% and 0.5 % of carbomer showed the best physical stability results and safe for topical preparations. It can be concluded that the peel-off mask gel prepared from MN leaves extract is potential for preventing hyperpigmentation as well as safe to be used as a topical preparation.

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