

RESEARCH ARTICLE

Peel-off gel formulation from black mulberries (*Morus nigra*) extract as anti-acne mask

Arif Budiman¹, Diah Lia Aulifa², Arif Satria Wira Kusuma¹, Insan Sunan Kurniawan¹, Astri Sulastri¹

¹Department of Science and Technology, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Kabupaten Sumedang, Jawa Barat, Indonesia, ²Department of Biology Pharmacy, Sekolah Tinggi Farmasi Indonesia, Bandung Kidul, Kota Bandung, Jawa Barat, Indonesia

Correspondence to: Arif Budiman, E-mail: arifbudimanapt@gmail.com

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ABSTRACT

Background: Acne is a skin disease characterized by chronic inflammation in the polisebasea that often occurs in adolescence. Utilization of the black mulberry fruit with a high phenolic is one alternative for the treatment of acne. **Aims and Objectives:** The aims of this research are to develop and test a peel-off mask gel prepared from black mulberries (*Morus nigra*) extracts, which has antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes*. **Materials and Methods:** Black mulberry fruit was extracted with the maceration method by using ethanol (96%). Then, the antibacterial activity of the extract was determined by the disc-diffusion method, while the mean inhibitory concentration (MIC) and mean bactericidal concentration (MBC) were determined by the microdilution method. Then, the extract was formulated into the base of the peel-off mask gel containing variations in concentration of polyvinyl alcohol (7%, 9%, and 10%) and hydroxypropyl methylcellulose (2% and 2.5%). The formulations prepared were evaluated for their physical properties, including organoleptic behavior, homogeneity, pH, viscosity, spreadability, and drying time. Irritation tests were performed, and antibacterial activity of the formulation was also assessed. **Results:** The results showed that the black mulberry fruit extract has antibacterial activity with MIC value of 2.5% against *S. epidermidis* and *P. acnes*, while MBC values were 2.5% and 5%, respectively. Formulations with the best results of physical evaluation were obtained for the formula containing polyvinyl alcohol at 7% and hydroxypropyl methyl cellulose at 2.5%. The antibacterial activity of the peel-off mask gel formulation from black mulberries (*M. nigra*) extract occurs with 3 × MIC and produce inhibition zone 6.11 ± 3.2 mm against *S. epidermidis* and 5.43 ± 0.6 mm against *P. acnes*. **Conclusion:** It can be concluded that the peel-off mask gel prepared from black mulberry (*M. nigra*) fruit extract is effective as well as safe to be used as a topical preparation.

KEY WORDS: *Morus nigra* Extract; Peel-off Mask Gel; Antiacne; *Staphylococcus epidermidis*; *Propionibacterium acnes*

INTRODUCTION

Acne is an chronic inflammatory skin disease that affects the skin's sebaceous glands and often occurs in the post-puberty

age.^[1,2] According to Global Burden of Disease, acne vulgaris affects approximately 85% of teenagers and young adults, i.e., the age range of 12-25 years.^[3]

One of the main causative factors of acne is the bacterial activity on the skin surface. *Propionibacterium acnes* and *Staphylococcus epidermidis* are skin microbiota that is often isolated from acne lesions and are known to contribute to the pathogenesis of acne.^[4] The previous study shows that *P. acnes* and *S. epidermidis* have resistance and low sensitivity toward several antibiotics.^[5,6] Natural plant sources have been used since centuries in Indonesia to overcome various health issues.

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One of the natural herbs that act as an antibacterial agent is black mulberry (*Morus nigra*) because of its high content of phenolic compounds as compared to other plants of genus *Morus*.^[7,8] There are several compounds extracted from black mulberry that possesses antibacterial activity, including 1-arylbenzofuran (Moracin M), stilbenoid oxyresveratrol, morusin, and kuwanon C.^[9,10] Due to these constituents, the black mulberry (*M. nigra*) has potential as an anti-acne agent for facial skin treatment.

The peel-off face mask gel is one of the popular forms of topical applications used to enhance the quality of the facial skin.^[11,12] The skin face mask has advantage of being easily peeled off or removed as an elastic membrane.^[13] The peel-off gel face mask is useful to recover/fix and treat the facial skin and can be used to minimize pores.^[14] In addition, it is also useful for relaxing the muscles of the face and as a cleanser or freshner.^[12]

With these considerations, this study was aimed at developing and testing a peel-off face mask gel from black mulberries (*M. nigra*) extract, which possesses antibacterial activity as an alternative facial skin care product.

MATERIALS AND METHODS

Materials

The materials used in this study consists of black mulberry (*M. nigra*) obtained from plantation in Cibodas, Maribaya-Lembang, glycerin, hydroxypropyl methylcellulose (HPMC), Mueller-Hinton Agar (MHA), Mueller-Hinton Broth, 0.9% NaCl, ethanol 96%, polyvinyl alcohol (PVA), sodium dodecyl sulfate (SDS), dimetil sulfoksida (DMSO), *P. acnes* and *S. epidermidis* from Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran.

Extraction

For preparation of the gel, we first obtained 10 kg of black mulberry and dried it by incubation in the oven at 50°C. For extraction, the dried fruit was macerated with 96% ethanol at room temperature. Ethanol was then removed by using a rotary evaporator with a vacuum pressure of 50°C to obtain a crude extract.^[15,16]

Black Mulberries Extract Phytochemical Screening

The crude ethanolic extracts of black mulberry (*M. nigra*) were tested for the presence of alkaloid, steroid/triterpenoid, saponin, polifenol, tannin, flavonoid, kuinon, monoterpenoid, and sesquiterpenoid.

Antibacterial Activity Test on Black Mulberries Extract

Antibacterial activity of this extract was then tested with the disc-diffusion method. MHA was used for *S. epidermidis* and

P. acnes media. The crude extract was dissolved in DMSO 0.01% at various concentrations: 10%, 20%, 30%, 40%, and 80%. Paper discs (6 mm diameter) were soaked in 5 mL of the extract solution for 15 min and then dried in a laminar air flow cabinet for 2 h. The paper discs were then placed on the media surface that been inoculated with the bacteria. Petri dishes were incubated at 37°C for 18 h. Tests were performed with three repetitions.^[17,18]

Determination of Mean Inhibitory Concentration (MIC) and Mean Bactericidal Concentration (MBC) of Extract

The MIC and MBC of black mulberry extract were determined using the micro-well dilution method with a microplate with wells.^[19] MHA medium (100 µL) was pipetted into the wells to which 100 µL of the extract was added. The extract was diluted by pipetting 100 µL from the first well to another well. Then, to each well, 10 µL of the bacteria suspension was added with 0.5 McFarland turbidity. Next, the microplate was covered with wrap plastic and then incubated at 37°C for 18 h. The incubation results were subcultured in MHA medium. MIC was determined as the lowest concentration from the extract that can inhibit bacteria growth. On the other hand, MBC was determined as the lowest concentration of the extract that could kill the bacteria.

Peel-off Mask Gel Formulation from Black Mulberry Extract

The formulation of peel-off mask gel from black mulberry extract was made according to the formula presented in Table 1.

PVA was mixed with distilled water (80°C) with constant stirring using a mechanical stirrer, and then HPMC was dispersed into the PVA solution. Extract and preservatives were dissolved in glycerol before being added to the PVA and HPMC solution. The mixture was then stirred until homogenous. Physical stability of peel-off mask gel formulation from black mulberry extract was evaluated through organoleptic, homogeneity, pH, and viscosity until 28 days.^[13,20,21]

Table 1: Peel-off mask gel formulation from black mulberry extract

Material	Formula (%)					
	I	II	III	IV	V	VI
PVA	7	9	10	7	9	10
HPMC	2	2	2	2.5	2.5	2.5
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
NaOH	Qs	Qs	Qs	Qs	Qs	Qs
Berrys Essence	Qs	Qs	Qs	Qs	Qs	Qs
Aquadest Ad (mL)	100	100	100	100	100	100
Black mulberry extract	2.5	2.5	2.5	2.5	2.5	2.5

Drying Time

The gel (0.7 g) was placed over the glass plates 7.0 cm × 2.5 cm and then spread flat. The glass plate was then placed in the oven at 34.5°C-38.5°C temperature for 1 h. Then, the gel was observed until it was completely dry and easily peeled off as a film layer. Drying time was evaluated until 28 days.^[12]

Coverage Test

Coverage test was then performed by weighing 0.5 g of the gel, placed on a 20 cm × 20 cm glass and then covered with another glass of the same size. Weights up to 125 g were placed on top, and after 1 min, the diameter was measured coverage test was evaluated until 28 days.^[13]

Antibacterial Activity Test on Peel-off Gel Mask from Black Mulberry (*M. nigra*) Extracts

Antibacterial activity of the peel-off gel face mask was tested with the disc diffusion method. Paper discs (6 mm diameter) were soaked in 5 mL of the sample solution for 15 min. The paper discs were then placed on MHA media surface that been inoculated with bacteria. Positive control contained only MHA medium with the bacterial suspension, while the negative control contained only MHA medium. Petri dishes were then incubated at 37°C for 18 h.

Irritation Test on Peel-off Mask Gel from Black Mulberry (*M. nigra*) Extracts

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). The irritation test was performed on three volunteers with the following criteria: (A) Inclusion criteria for the female volunteers were as follows:^[22,23] (i) Age 18-24 years, (ii) no history of allergy-related diseases, and (iii) provided informed consent for participation. (B) Exclusion criteria as follows:^[24] (i) Presence of any lesions, marks such as tattoos, or scars on the test area, (ii) irritation of the skin in the test area, (iii) use of drugs (hypo-allergenic or anti-inflammatory), which can affect the skin, especially the response to the test area, (iv) skin disease, and (v) participants of other studies. The irritation test was performed using the repeated patch test method. The inner aspect of the arm was cleaned with alcohol 70%, and then the gel and base was applied at 1 g each on the cleaned area (3 cm × 3 cm). SDS 0.5% was used as the positive control and aqua dest as the negative control. The test was performed with 4 applications over 24 h. The evaluation test was done at 15 min, 1 h, and 24 h after the gel application was cleaned (Table 2).^[25]

Data Analysis

The data obtained from physical evaluation, irritation test, and product preference test were statistically analyzed using

analysis of variance (ANOVA) method. If the data were not normally distributed, then the Kruskal–Wallis analysis method was used with the Statistical Product and Service Solution software version 21.

RESULT

Plant Determination

Plant determination tests were done at the Department of Biological Science, UNPAD, showed that the plant used was *M. nigra* L.

Phytochemical Screening

The results of phytochemical screening showed that the ethanol extract of black mulberry fruit has potential antibacterial properties. The screening results are shown in Table 3.

Antibacterial Activity of Black Mulberry (*M. nigra*) Fruit Extract

The results of the antibacterial activity test (Table 4) show that the black mulberry fruit ethanol extract can inhibit the growth of bacteria *S. epidermidis* and *P. acnes*.

MIC and MBC Black Mulberry (*M. nigra*) Fruit Extract

The results of subculture revealed that the MIC value of the ethanol extract of black mulberries against *S. epidermidis* bacteria was 1.25-2.5%, while that for *P. acnes* was 2.5-5% (Table 5).

Table 2: Response and irritation index's categories^[26,27]

Categories	Irritation index
Not significant (no erythema)	0-0.4
Low irritation (slight erythema)	0.5-1.9
Moderate irritation (erythema was evident)	2-4.9
Severe irritation (moderate to severe erythema)	5.0-8.0

Table 3: Results of phytochemical screening ethanol extract of black mulberry (*M. nigra*)

Phytochemical screening	Result
Alkaloid	-
Flavonoid	+
Polifenol	+
Tanin	+
Monoterpenoid dan seskuiterpenoid	+
Steroid dan triterpenoid	-
Kuonon	-
Saponin	-

-: Not detected, +: Detected, *M. nigra*: *Morus nigra*

Organoleptic and Homogeneity

During the storage period, it does not change color, smell, or homogeneity. Homogeneity of the preparation was shown by the absence of coarse particles in the preparation after its application to a transparent glass.^[23] However, FI showed fungal growth on day 28 and the consistency become watery on storage. This may have been caused by the process of syneresis, which is one of the characteristics of gel formulations. High water levels in syneresis process cause the stocks to become vulnerable to microorganism overgrowth. On day 28, FI could not be evaluated.

pH

For topical preparations, the pH should be in the range of skin pH (4.5-6.5) to avoid any irritation to the skin(Figure 1).^[13]

Table 4: Results of antibacterial activity test ethanol extract of black mulberry (*M. nigra*)

Extract concentration (% b/v)	Inhibition zone diameter (mm)	
	<i>S. epidermidis</i>	<i>P. acnes</i>
10	6.50±2.19	0
20	12.28±2.82	5.81±2.64
40	15.33±0.61	11.28±1.29
80	19.46±1.86	15.12±1.50
Solvent control DMSO 0.01%	0	0

DMSO: Dimetil sulfoksida, *S. epidermidis*: *Staphylococcus epidermidis*, *P. acnes*: *Propionibacterium acnes*, *M. nigra*: *Morus nigra*

Table 5: Results of subculture determination MIC and MBC determination ethanol extract of black mulberry (*M. nigra*)

Extract concentration (% b/v)	<i>P. acnes</i> bacterial growth	<i>S. epidermidis</i> bacterial growth
20	-	-
10	-	-
5	-	-
2.5	+	-
1.25	+	+
0.625	+	+
0.3125	+	+
0.156	+	+
0.078	+	+
Media control MHB	-	-
Bacterial control	+	+
Extract control	-	-

MIC: Mean inhibitory concentration, MBC: Mean bactericidal concentration, *S. epidermidis*: *Staphylococcus epidermidis*, *P. acnes*: *Propionibacterium acnes*, *M. nigra*: *Morus nigra*

Viscosity

As shown in Figure 2, the greater the concentration of HPMC as the gelling agent, the higher was the viscosity of the preparation. This can occur because of greater retention and binding of the fluid by the gelling agent; therefore, the viscosity of the preparation increases.

Dispersion Test

The dispersion test was performed to examine the ability of the preparations to spread and to evaluate the ease of application during the storage period. A good peel-off mask gel has a dispersion of 5-7 cm.^[13]

Drying Time

Ideally, the peel-off face mask should dry at 15-30 min.^[14]

Antibacterial Activity Test of Peel-off Gel Mask Black Mulberry (*M. nigra*) Fruit Extract

The result of antibacterial activity test of peel-off gel mask black mulberry (*M. nigra*) fruit extract can be seen in Table 6.

DISCUSSION

Drying and Extraction

The drying temperature was adjusted such that the compounds contained in the black mulberries were not destroyed. Purplish black color from black mulberry fruit was due to anthocyanin compound; this compound is stable in an acidic environment (pH <5) and 50°C; therefore, the drying temperature was not over 50°C.^[28] From the drying process, 846,86 g of black mulberries was obtained.

The extraction was performed by maceration, which aimed to protect the compounds contained in black mulberry fruit from degradation, especially the thermolabile compounds such as anthocyanin. The solvent used for the maceration process was 96% ethanol because it is universal, it can dissolve polar and nonpolar compounds, and it is safe for topical application. The expected compound that was extracted in the maceration process was phenol since the compounds were responsible for the cause of antibacterial activity. Studies by Suryani^[29] showed that the higher ethanol concentration; the higher is the levels of phenol obtained. According to Lee et al.,^[30] ethanol extracts have with higher phenol levels than water extracts. Maceration products then evaporated to obtain a thick extract. The rendement of extract was 44 417%.

Phytochemical Screening

Phytochemical screening showed that the ethanol extract of black mulberry (*M. nigra*) contains flavonoid, polyphenol,

Table 6: Results of antibacterial activity test peel-off mask gel black mulberry fruit extracts (*M. nigra*)

Formula	Inhibition zone (mm)	
	<i>S. epidermidis</i>	<i>P. acnes</i>
Basis	0	0
FI	0	0
FII	6.11±3.2	5.43±0.6
FIII	6.83±1.4	6.76±0.9

FI: Formulation consists of PVA concentration of 7%, 2.5% HPMC and 1×MIC extract, FII : Formulation consists of PVA concentration of 7%, 2.5% HPMC and 3×MIC extract, FIII: Formulation consists of PVA concentration of 7%, 2.5% HPMC and 5×MIC extract. PVA: Polyvinyl alcohol, HPMC: Hydroxypropyl methyl cellulose, MIC: Mean inhibitory concentration, *S. epidermidis*: *Staphylococcus epidermidis*, *P. acnes*: *Propionibacterium acnes*, *M. nigra*: *Morus nigra*

tannin, monoterpene, and sesquiterpene compounds. Flavonoid, tannin, and polyphenols have been reported to have antibacterial activity.^[31-33]

Antibacterial Activity of Black Mulberry (*M. nigra*) Fruit Extract

The results of the antibacterial activity test (Table 4) show that the black mulberry fruit ethanol extract can inhibit the growth of bacteria *S. epidermidis* and *P. acnes*. The existence of secondary metabolites is an important factor of antibacterial activity. The compounds include flavonoid, polyphenol, and tannin. Flavonoid compounds can form hydrogen bonds with the protein in the bacterial cell wall, causing them to become instability and lose their biological activity.^[31] The antibacterial activity of phenol compounds is exerted through the denaturation bacterial cell proteins.^[33] Tannin also exerts its antibacterial activity by targeting polypeptide on the cell wall disrupting the formation of the cell wall and making it prone to lysis.^[31,32]

MIC and MBC Black Mulberry (*M. nigra*) Fruit Extract

The results of subculture revealed that the MIC value of the ethanol extract of black mulberries against *S. epidermidis* bacteria was 1.25-2.5%, while that for *P. acnes* was 2.5-5%. The MBC value of the ethanol extract of black mulberries was 2.5% against *S. epidermidis* bacteria and 5% against *P. acnes*. From these results, it can be concluded that the ethanol extract of black mulberries still provides antibacterial activity against *S. epidermidis* and *P. acnes* at a concentration of 2.5%, which can be used in the preparation of peel-off gel face mask.

Evaluation of Peel-off Mask Gel Black Mulberry (*M. nigra*) Fruit Extract

The gel form of the ethanol extract of black mulberries, which possess antibacterial activity, affords ease of use, longer contact time, high water content, and cooling sensation and also reduces the risk of inflammation.^[34]

pH

During storage, the pH decreased but still remained within the acceptable levels for topical preparations. Based on the results of statistical analysis by ANOVA, the value of significance was 0.020 ($P < 0.05$), which indicates that there is an effect of the amount of time on the pH values of the preparation. Based on research data, pH values of FV had the highest standard of deviation and its pH value was influenced by the length of time of storage.

Viscosity

Based on the results of ANOVA, the value of significance was 0.632 ($P > 0.05$), which indicated that there was no influence of the storage time on the preparation viscosity. From the data, it is clear that FI and FII had the lowest viscosity compared to other formulae because of low polymer content.

Dispersion Test

From Figure 3, the dispersion shows a decrease during the storage period. The decrease of the dispersion occurs through an increase in the size of the unit that absorbs the solvent molecules; therefore, the liquid was retained, with increased resistance to flow and spread.^[35] Based on the results of statistical analysis by the Kruskal–Wallis test indicated that the significant value was 0.039 ($P < 0.05$), indicating that there is an effect of the amount of time on the value of the dispersion of the preparation. The results of statistical analysis indicated that the change in the dispersion over time was the greatest for formula FII. Therefore, the dispersion value of FII was affected by the storage period. The dispersive value of FI-FII increased, while that of FIII-FVI decreased over time. Preparations with high concentrations of gelling agent and PVA become difficult to spread over time.

Drying Time

From Figure 4, it can be seen that during the storage period, the drying time of the preparations decreased. In the storage period, the liquid will be absorbed by the gelling agent causing an increase in the viscosity, which makes the drying time shorter. Statistical analysis by the Kruskal–Wallis test yielded a significance value of 0.001 ($P < 0.05$), indicating that there is an effect of the amount of storage time on the drying time of the preparations. Based on the results of statistical analysis, formula FII has the greatest change in the drying time. Therefore, the drying time for FII was most affected by the storage period. Thus, the observations and statistical analysis indicated that variation in the concentration of PVA and HPMC affected the drying time during storage.

Antibacterial Activity Test of Peel-off Gel Mask Black Mulberry (*M. nigra*) Fruit Extract

Antibacterial activity test of the preparation of peel-off gel mask was done formula IV (PVA, 7% and HPMC, 2.5%),

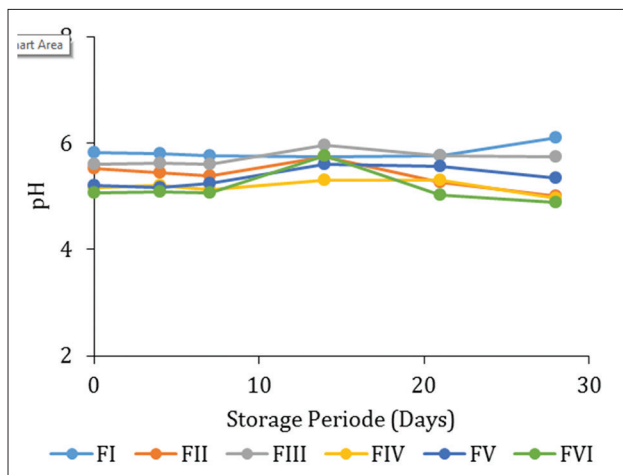


Figure 1: Graph measurements of pH during storage period

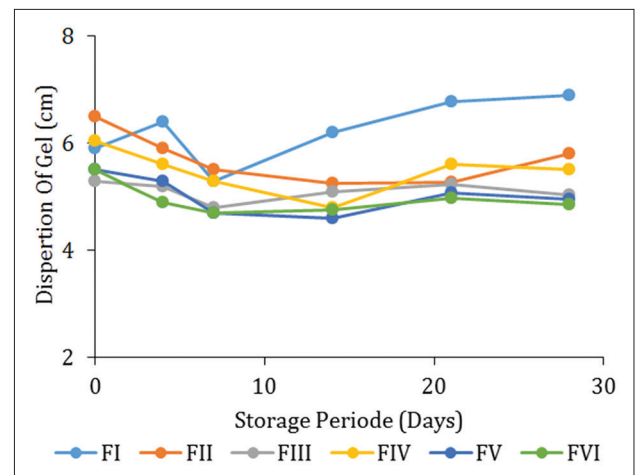


Figure 3: Graph measurements of dispersion test during storage period

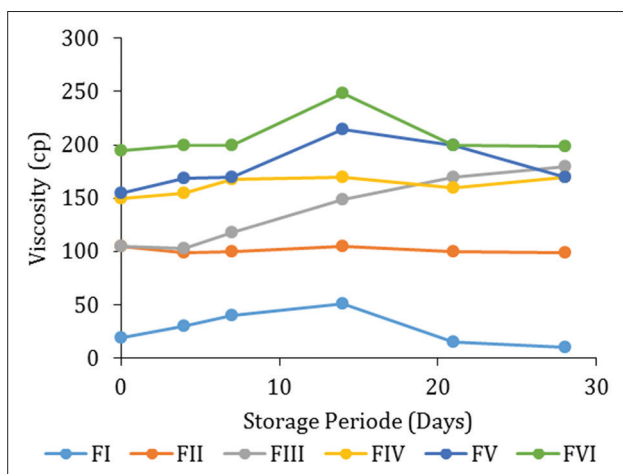


Figure 2: Graph measurements of viscosity period during storage

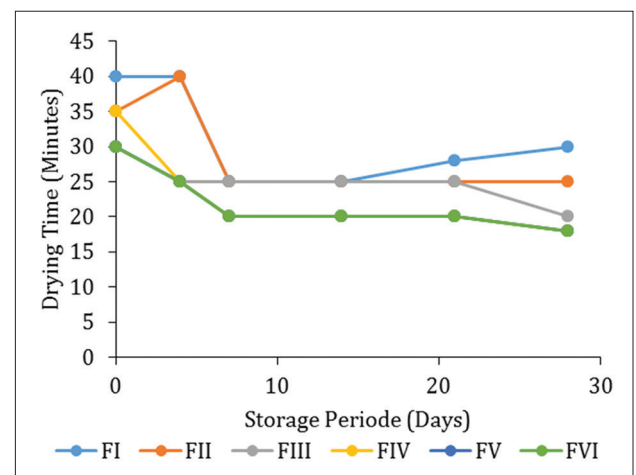


Figure 4: Graph measurements of drying time during storage period

which resulted in the best physical properties during the storage time.

The test results demonstrate that the antibacterial activity of the preparation in terms of the diameter of the inhibitory zone increased with an increase in the extract concentration in the preparation. The peel-off gel mask prepared with black mulberry fruit extract had antibacterial activity at a concentration of $3 \times$ the MIC of the extract. Further, the base of the peel-off gel mask did not show any antibacterial activity that may affect the results of the extract activity. The results of the antibacterial activity test are shown in Table 6.

The increase in the diameter of the inhibition zone in the preparation was suspected due to an increase in the antibacterial compound penetration that diffuses into the test media. Bacteria *S. epidermidis* and *P. acnes* are Gram-positive bacteria that have high levels of peptidoglycan, small amounts of lipids, and polysaccharides (teichoic acid) in their cell wall. Teichoic acid can transport positive ion and water-soluble polymer. The water-soluble of polymer shows that the cell wall of Gram-positive bacteria is highly polar.^[36] High water levels in gel formulation can increase

the penetration of antibacterial compounds in the preparation through the peptidoglycan layer, which is polar.

Irritation Test

Results of the statistical analysis by the Kruskal–Wallis test yielded a $P < 0.05$, suggesting an association the test material and longer observation time. The longer the observation time, the less visible were the effects of the test material.

Positive control of 0.5% SDS showed the highest irritation index, indicating low irritation levels, while the irritation index of preparation FIV indicated that it showed no significant reaction. The results of statistical analysis of the tested samples by the Kruskal–Wallis test indicated that the irritation index was 1:00 ($P > 0.05$), indicating that there was no significant reaction of the tested samples (base preparation, formula FIV, SDS 0.5%, and distilled water) during the observation duration. Therefore, it can be concluded that the peel-off mask gel prepared from black mulberry (*M. nigra*) fruit extract is safe to use as a topical preparation because it does not cause significant irritation reactions.

CONCLUSION

Extracts of black mulberry (*M. nigra*) have antibacterial activity against *S. epidermidis* with MIC value of 1.25-2.5% and MBC value 2.5%. Against *P. acnes*, the MIC and MBC values of the extract were 2.5-5% and 5%, respectively. In terms of the physical characteristics, the best peel-off mask gel formulation was obtained with 7% PVA and 2.5% HPMC. We found that peel-off mask gel containing black mulberries (*M. nigra*) extract had antibacterial activity at a concentration of 3× MIC, with an inhibition zone of 6:11 ± 3.2 mm against *S. epidermidis* and 5:43 ± 0.6 mm against *P. acnes*.

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