Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2018; 9(1); 95-101
doi: 10.25258/ijpqa.v9i01.11922

ISSN 0975 9506

Research Article

Development of Gel Dossage Form From Mulberry Fruit Extract as A Facial Treatment

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Received: 25th Sep, 17; Revised: 31st Dec, 17; Accepted:15th Mar, 18; Available Online: 25th March, 2018

ABSTRACT

Skin care is very important in cosmetics, especially facial treatments. Black mulberry is rich in phenols and is therefor usable in the treatment of acne. It also contains anthocyanin, a well-known antioxidant and a potential source of sun protection. This research aimed to develop a gel dossage from black mulberries (*Morus nigra*) extracts that would function as an antibacterial, antioxidant and sun blocking facial treatment. This research started with black mulberry fruit extracted through the maceration method by using ethanol (96%). Then, the antibacterial activity of the extract was determined by the disc-diffusion method, while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by the microdilution method. Antioxidant activity of the extract was determined using the 1,1-diphenyl-2-picrilhidrazil (DPPH) method with vitamin C as a comparison. Furthermore, the extracts were formulated into gel formulas with variations of HPMC, Na-CMC, carbopol 934 and extract concentrations. The products were then physically evaluated for organoleptics, homogeneity, pH, viscosity, and dispersion as well as undergoing a hedonic test, an irritation test, an antioxidant activity test and determining the SPF value of the preparation. The results showed that the black mulberry fruit extract has antibacterial activity with MIC value of 0,025 g/mL against *S. epidermidis* and *P. acnes*, while MBC values were 0,025 g/mL and 0,05 g/mL, respectively. The black mulberry extract had antioxidant activity with IC_{50} value, i.e 146,73 µg/mL vitamin c i.e. 3,17 µg/mL. Formulation with best physical evaluation results showed ina formula containing a carbopol gel base of 934 0,015 g/mL with an extract cconcentration of 0,075 g/mL. This formula resulted in antioxidant activity with an IC_{50} value, i.e 1004,6 µg/mL, antibacterial activity with inhibition zone 6.83 ± 1.4 mm against and 6.76 ± 0.9 mm against *S. epidermidis* and *P. acnes* respectively and an SPF value of 1.9.

Keyword : Black mulberry, gel, antibacterial, antioksidant, SPF

INTRODUCTION

Our skin protects us against environmental influences such as the ultraviolet rays of the sun, microbes or pollution. Environmental effects can cause damage to the skin such as premature aging and acne. Premature aging is usually caused by frequent exposure to ultraviolet light, air pollution and poor lifestyle [1,2]. Ultraviolet light can cause sunburn and can trigger free-radical formation faster, thus accelerating skin damage, esspecially premature aging [3]. Acne is a chronic inflammatory skin disease that affects Sebaceous skin glands[4,5]. One of the main causes of acne is bacterial activity on the surface of the skin such as *Propionibacterium acnes* and *Staphylococcus epidermidis*, so preventing skin damage is one reason for using antiacne [6,7,8]. Some of the subtances commonly used in cosmetic preparations are tocopherol, ascorbic acid, vitamin A, Antibiotic, and zinc[9]. However, the use of synthetic subtances can be carcinogenic and toxic in high doses [10]. Natural antioxidants are used as an alternative; one of which is the black mulberry. This plant contains the highest phenolic and flavonoid compounds as compared to other mulberry plants, and has potential antioxidant activity called anthocyanin[11,12,13]. These anthocyanin compounds are cyanidin-3glucoside and cyanidine-3-routoside [14]. Additionally, there are several compounds extracted from the black mulberry that possess antibacterial activity, including 1-arylbenzofuran (Moracin M), stilbenoid oxyresveratrol, morusin, and kuwanon C [13,15]. Due to these constituents, the black mulberry (*M. nigra*) has potential as an anti-acne agent for facial skin treatment. This research is aimed to develop a gel dossage form from black mulberries (*Morus nigra*) extracts, which has antibacterial activity, antioxidant and sun protector as facial treatments. The extract of black mulberry was preferred in a gel form because it is easy to spread, easy to wear and comfortable on the skin [16].

MATERIAL AND METHODS

The materials used in this study consists of black mulberry (*M. nigra*) obtained from a plantation in Cibodas, Maribaya- Lembang, Mueller-Hinton Agar (MHA) (Merck Ltd), Mueller-Hinton Broth (Merck Ltd), 0.9% NaCl, 96% ethanol, polyvinyl alcohol (PVA), sodium dodecyl sulfate (SDS) (Merck Ltd), dimetil sulfoksida

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(DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), glycerin (Brataco), hyper methyl cellulose (Cognis), carbopol 934 (Cognis), methyl paraben (Brataco), sodium carboxy methyl cellulose (Brataco), propyl paraben (Brataco), triethanolamine (Sarana Abdi Bakti), and vitamin C (Merck Ltd), *P. acnes* and *S. epidermidis* from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran.The tools used in this study include an incubator (Yenaco), a magnetic stirrer (Yellow-MAG HS7), a mechanical stirrer (IKA EUROSTAR), a micropipette (Socorex), ovens, pH meters (108 pH ATC), an analytical balance (OHAUS TM-Adventure), a Viscometer Rion (VT 04F), and a UV-Vis Spectrophotometry Analytik (Jena Specord).

Extraction

Ten kilograms of black mulberry were extracted and then dried by incubation in the oven at 50°C for 3x24 hours (Chen et al., 2016). The dried fruit was macerated with 96 % ethanol at room temperature The extract was thickened using using a rotary evaporator with a vacuum pressure of 50°C[17,18].

Black Mulberries Extract Phytochemical Screening

The phytochemical screening of black mulberry fruit extracts was performed based on the Fansworth method including the examination of alkaloids, flavonoids, polyphenols, tannins, saponins, quinones, steroids / triterpenoids, monoterpenoids and sesquiterpenoids [19].

Antibacterial Activity Test on Black Mulberries Extract

The antibacterial activity of the extract was tested using the disc-diffusion method with MHA being 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 two hours. The paper discs were then placed on the media surface that been inoculated with the bacteria. Petri dishes were incubated at 37°C for eighteen hours[20,21].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum 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 An MHA medium (100 µL) was pippeted into the wells to which 100 µL of the extract was added. The extract was diluted by pippeting 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 plastic wrap and then incubated at 37°C for eighteen hours [22].

Antioxidant Activity Test of Black Mulberry Fruit Extract

A DPPH solution (20 ppm) was prepared using 96 % ethanol as a solvent. Then the λ_{max} DPPH and DPPH operating time for one hour with a five-minute interval was determined. Extracts and vitamin C (reference) as stock solution (1000 ppm) were prepared. 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 concentration and mixed with a DPPH solution (2:3), stored during operating time and then absorbance was measured at λ_{max}. The values of absorbance are calculated for the value of the percentage inhibition by using the equation:

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

Where, % Inhibition is the percentage capacity of free radical inhibition, A_{sample} is the absorbance of the sample, and A_{DPPH} is the absorbance of DPPH control.

The IC50 value of the sample was obtained by entering a value of 50 into the equation of each sample [19].

Formulation of Antioxidant Gel from Black Mulberry Fruit Extract

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

Evaluation of antioxidant gel formula

The formula including organoleptic, homogeneity, pH and viscosity were observed for 28 days.

Spreadability

One gram of gel was placed in the center of the glass (20 cm x 20 cm) and then covered with another glass, a weight of 125 g was then added and after a minute the diameter of the gel distribution was measured [23].

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

The antibacterial activity of the gel was tested with the disc diffusion method. Paper discs (6 mm diameter) were soaked in 5 mL of the sample solution for fifteen minutes. The paper discs were then placed on an MHA media surface that had been inoculated with bacteria. Positive control contained the MHA medium with the bacterial suspension, while the negative control contained only the

Table 1. Formula 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	-	-	-	-	-	-
HPMC	-	-	-	2	3	4	-	-	-
Sodium-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	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %
Aquadest ad	100	100	100	100	100	100	100	100	100

MHA medium. Petri dishes were then incubated at 37°C for eighteen hours[22].

Antioxidant Activity Test of Black Mulberry Fruit Extract in Gel

Gel was dissolved in 10 mL of a 96 % ethanol solution and then synthesized for twenty minutes and centrifuged for ten minutes at 5000 rpm. Then the sample solution was mixed with a DPPH solution (2:3), stored during operating time. Furthermore, the absorbance at λ_{max} was measured and the percentage inhibition value was obtained. The linear regression curve was plotted and linear equation and IC_{50} value were obtained

SPF-value Tests of Antioxidant Gel

This test was refers to Khan[24] modifications. Exactly 0.5 g of each formula was dissolved in 10 mL of a 96 % ethanol solution and then synthesized for twenty minutes and centrifuged for ten minutes at 5000 rpm. Then the absorbance of the solution was measured at a wavelength of 290-320 nm with a 5 nm interval. The absorbance value was fed into the Sayre equation to obtain the SPF value as follows:

$$SPF_{spectrofotometer} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF is the correction factor (10), EE (Erythermal Effect) is the arithmogenic effect of radiation with wavelength (λ), Abs is the spectroscopy of absorbance photometry at wavelength (λ), and I is the intensity of the light spectrum. The values of EE x I are constants.

Table 3. EE x I Value (Khan, 2014)

Wavelength	EE x I value
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

The EE x I value was represented by the table above, then put into the equation. The result of the calculation becomes the SPF-value of each formula with the following parameters:

Table 1. SPF Value (Miller, 2005)

SPF Value	Parameters
2-11	minimum strength
12-29	medium strength
>30	maximum strength

RESULTS AND DISCUSSION

Plant Determination

The plant was taxonomicaly identified by a professor of biology in the Faculty of Science at Padjadjaran Uniersity and identified as *Morus nigra* L.

Extraction

The drying process using a temperature 50^{o}C to maintain the stability of anthocyanins and prevent fungus growth because the water content of black mulberry is very high (85,5 %) and the pH is conducive to fungus growth (pH 5)[25]. The anthocyanins stable at low temperature and the purple color was faded at temperatures greater than 80°C. Ethanol 96 % is the solvent that can attract the highest concentration of anthocyanin and is often used to attract antioxidant compounds in the fruit [26,27,28]. The thickening of the extract was performed using a rotary evaporator because the anthocyanin compound is thermolable. However, there was still anthocyanin degradation in this process, gradually removing the purple color [29,30].

Phytochemicals Screening of Extracts

The results of a phytochemical screening of the *Morus nigra* extract can be seen in Table 5.

Table 5. Phytochemicals Screening of Black Mulberry Extracts

Compound	Results
Alkaloids	-
Flavonoids	+
Saponins	-
Polyphenols	+
Mono and seskuiterpenoid	+
Tanins	+
Quinons	-
Steroids/triterpenoids	-

Where, (+) presence and (-) absence

Based on a phytochemical screening, the extracts were detected to contain flavonoid compounds, polyphenols, tannins, monoterpenoids and sesquiterpenoids. The black mulberry fruit is rich in flavonoids and phenols, i.e anthocyanin compounds which have potestial as antioxidant and antibacterial [28,31].

Antibacterial Activity Test of the Black Mulberry Extracts

Table 6. Result of Antibacterial Activity Test of Black Mulberry Extract

Extract conc. (% b/v)	Inhibition zone diameter (mm)	
	S. epidermidis	*P. acnes*
10	6.50 ± 2.19	0
20	12.28 ± 2.82	5.81 ± 2.64
Control (DMSO 0.01%)	0	0

The extract of *Morus nigra* could inhibit the growth of *S.epidermidis* and *P.acnes* bacteria. The presence of secondary metabolite compounds like flavonoids, polyphenols and tannins becomes an important factor in generating antibacterial activity. Flavonoids can form hydrogen bonds with proteins in bacterial cell walls, causing the instability of cell walls and the consequent loss of the bacteria's biological activity. Phenols can produce antibacterial activity through the denaturation of bacterial cell proteins because phenols are toxic for bacteria or disinfectant. Tannins produce antibacterial activity by targeting the cell-wall polypetides so that the formation of cell walls becomes imperfect and susceptible to lysis [32].

Determination of the MIC and MBC of Black Mulberry Extracts

Table 7. Results of MIC and MBC Tests of Black Mulberry Extracts

Extract concentration (g/mL)	Growth of *P. acnes*	Growth of *S. epidermidis*
0,2	-	-
0,1	-	-
0,05	-	-
0,025	+	-
0,0125	+	+
Media control	-	-
Bacteria control	+	+
Extract control	-	-

MIC values of black mulberry extracts against *S. epidermidis* bacteria was 0,0125–0,025 g/mL (b/v), while MIC value for *P. acnes* bacteria was 0,025–0,05 g/mL (b/v). The MBC value of black mulberry extracts against *S. epidermidis* bacteria was 0,025 g/mL (b/v), while the MBC value for *P. acnes* bacteria was 0,05g/mL (b/v).

Antioxidant Activity Test of Black Mulberry Fruit Extract

The IC_{50} of a vitamin C solution was 3,17 µg/mL. According to Jun et al. (2006), IC_{50} <50 µg/mL is classified into very powerful antioxidants. Vitamin C has four hydroxyl groups so that the antioxidant activity of vitamin C is strong enough to make DPPH become a stable radical. While the IC_{50} of the extract solution of black mulberry fruit is 146,73 µg/mL According to Jun et al. [33], the IC_{50} solution between 100–250 µg/mL is classified into medium antioxidants. According to McGhie et al.[34], the functional group examination using NMR spectrophotometry showed that the compound has many hydrogen atoms so that the compound can easily capture single electrons in free radicals.

Organoleptic and Homogeneity

Based on observation, the extract affected the organoleptic in terms of color. The colors darken due to the increasing concentration of the variation of gel-base. Carbopol has a

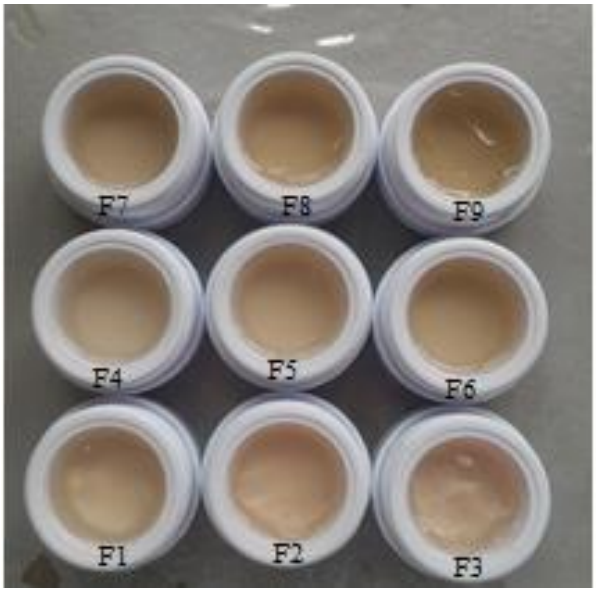

Figure 2. Gel of Black Mulberry Extracts

transparant color, HPMC has a cloudy color and Na-CMC has a faded-yellow color.

This occurs because high concentrations of HPMC made some parts of the intramolecular interaction in the polymer region of the matrix fibers rearrange the structure to produce a more compact gel and made the water squeeze out of the gel. This synthesis may occur due to both pH and temperature factors [35].

Measurement of pH

A good pH for the formula corresponds to the pH range of skin 4.5–6 [23]. During storage time, there was not a significant changed of pH, and the extract remained in the pH range of the skin.

Viscosity

Viscosity is affected by long storage periods. The increased viscosity will affect the dispersion and release of the active substance in the formula [36]. Based on figure 4, there was no significant difference during 28 days of storage. However, the formulas tend to increase in viscosity. Each base has a different viscosity even though the concentration was almost the same due to the structure of each gel-base. The more hydroxyl groups (-OH) on the

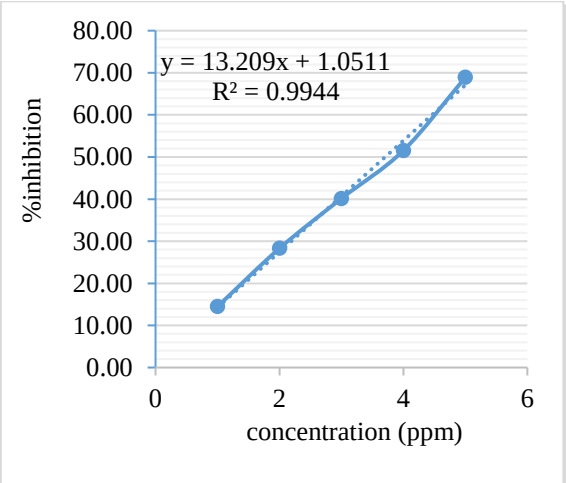

Figure1: (A) Graphic percentage inhibition and concentration of vitamin C

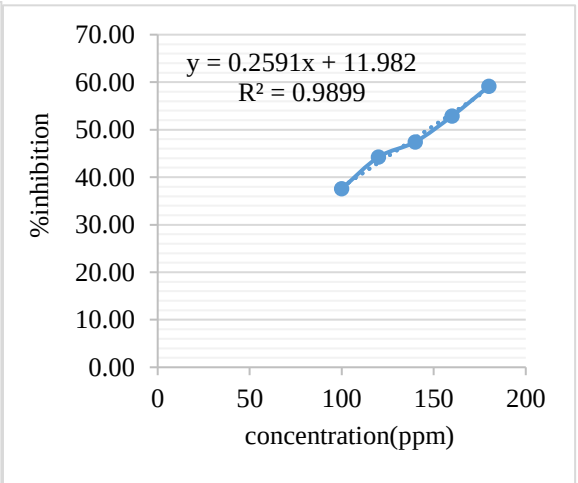

Figure 1: (B) Graphic percentage inhibition and concentration of black mulberry extracts

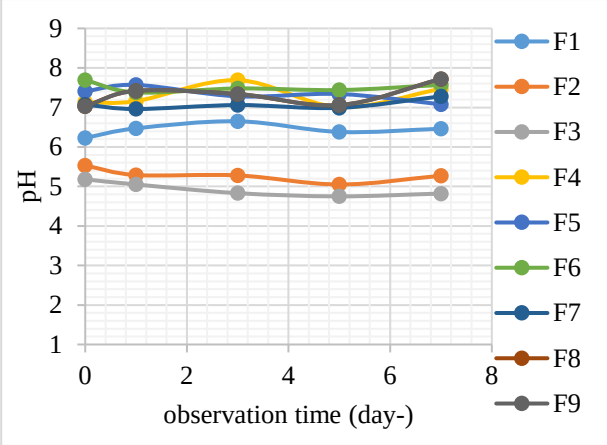

Figure 3. Graphic of pH Evaluation

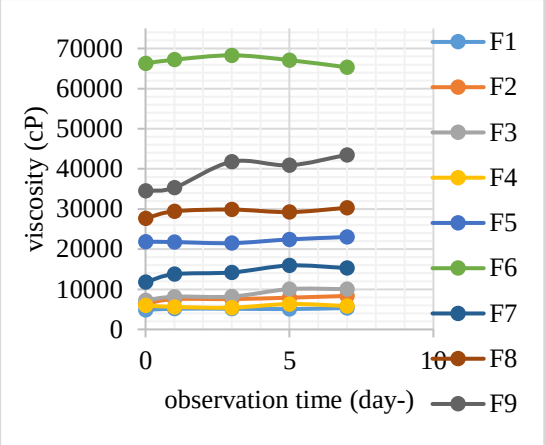

Figure 4. Graphic of viscosity evaluation

gel-base, the higher the viscosity. This hydroxyl group will form hydrogen bonds with water molecules that play a role in the hydration process during the swelling process. The optimum viscosity will retain the active substance and maintain the uniformity of the concentration of the base [37].

Spreadability

The dispersion in the formula that contains the active compound, such as the antioxidant gel, plays the important role of the absorption medium in the release of the active substance. The good scattering requirements for the formulas are 5–7 cm [23].

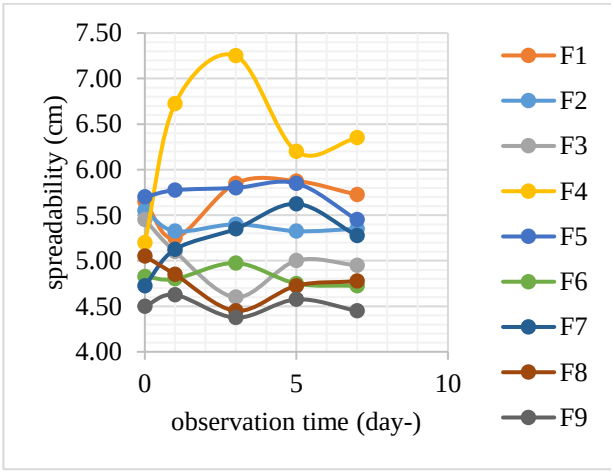

Figure 5. Graphic of spreadability evaluation

Spreadability is inversely proportional to viscosity. Increased viscosity will cause the decrease of dispersion[38]. Spreading will decrease as the gel-base concentration increases in the formula. Factors affecting the dispersion in the gel formula are the amount and strength of the gel-base. The higher concentration of the gel-base, the power of spreading will decrease and strengthen the gel-base. Formula containing Carbopol 934 with concentration of 1.5% was the best and most optimum concentration for gel formula. Carbopol gives a cold sensation, leaves no stickiness and does not cause the separation of phases (syneresis) [39,40].

Antibacterial activity test of black mulberry fruit extract gel

The results showed that the diameter of the inhibition zone was higher with the increasing of extract concentration in the formula. The diameter of the inhibition zone allegedly increased because the penetration of antibacterial compounds that diffuse into the medium test increased as well. The higher water content in gel was thought to increase the penetration of the compounds to the cell bacteria. *S.epidermidis* and *P.acnes* bacteria are gram-positive bacteria that have cell wall structures that contain polysaccharides (teicoic acid) with more peptidoglycan and fewer lipids. The teicoic acid functions as a positive ion transport and a water-soluble polymer. This water-soluble nature shows that gram-positive bacterial cell walls are more polar (so the compounds can penetrate the polar peptidoglycan layer more fully[19].

Table 8. Result of the Antibacterial Activity of Gel Black Mulberry Extracts

Formula	Inhibition Zone (mm)	
	S.epidermidis	*P. acnes*
Gel-base	0	0
F1	0	0
F2	6.11 ± 3.2	5.43 ± 0.6
F3	6.83 ± 1.4	6.76 ± 0.9

F1:Formula with 1x MIC extract concentration, F2 : Formula with 3x MIC extract concentration, F3: Formula with 5x MIC extract concentration

Antioxidant Activity Test of Black Mulberry Extract Gel

Based on an antibacterial activity test, formula 2 was selected for antioxidant analysis. F_2 was the minimum concentration of extract (7,5 %) that could present antibacterial activity. The results showed that IC_{50} of F_2 was 1004,2 ppm, while the gel-base didn't show any activity. According to Jun et al.[33], IC_{50} > 500 ppm is classified into powerless antioxidants. The anthocyanin as an antioxidant agent undergoes decreased stability. The formulas are stored in gel containers at room temperature while antioxidant compounds, i.e anthocyanins were stable in dark places and at cold temperatures (refrigerator temperature). Factors that affect the stability of anthocyanins during storage are pH, temperature, light,

and oxygen [41]. Although antibacterial activity was seen in the 7,5 % extract concentration, the antioxidant activity is classified as powerless. The active compound contained in black mulberry extracts acting as antibacterials are different from the active compounds that act as antioxidants. According to the literature, the compound on the black mulberry that has known antibacterial activity against *Streptococcus faecalis* (MBC 500 µg/mL) is 2-arylbenzofuran (Moracin M) and the compound that acts against *Staphylococcus aureus* (MBC 125 µg/mL) is oxyresveratrol stilbenoid (Majinda et al., 2011), while those working as antioxidants are cyanidin-3-glucoside dan cyanidin-3-rutinoside [42,43].

SPF-value Tests of Antioxidant Gel

Topical antioxidants should have at least SPF values in order to better protect the skin from UV rays [43]. The designation of sunscreen is indicated by the value of SPF (Sun Protection Factor). According to research Alhabsyi et al showed a positive relationship between antioxidant activity with SPF values. The greater the antioxidant activity, the greater the SPF SPF value. Based on the result of statistical analysis using one-way ANOVA showing a significant value of 0,115 ($p > 0,05$), H_0 was accepted, meaning that there is no difference of SPF value at various storage times. This indicates that there is no effect on SPF value during storage.

Table 9. SPF Value of Antioxidant Gel Formulas

SPF value	Day -	Formula -			
		1	2	3	4
	1	-	0.9808	1.2387	1.9179
	14	-	0.8615	0.9860	1.8475
	28		0.8468	0.7718	1.877

CONCLUSION

The black mulberry fruit extract has antibacterial activity with MIC value of 0,025 g/mL against *S. epidermidis* and *P. acnes*, while MBC values were 0,025 g/mL and 0,05 g/mL, respectively. The black mulberry extract had antioxidant activity with IC_{50} value, i.e 146,73 µg/mL vitamin c i.e. 3,17 µg/mL. Formulation with best physical evaluation results showed ina formula containing a carbopol gel base of 934 0,015 g/mL with an extract cconcentration of 0,075 g/mL. This formula resulted in antioxidant activity with an IC_{50} value, i.e 1004,6 µg/mL, antibacterial activity with inhibition zone 6.83 ± 1.4 mm against and 6.76 ± 0.9 mm against *S. epidermidis* and *P. acnes* respectively and an SPF value of 1.9.

REFERENCES

1. Yaar, M & B. Gilchrest. *Aging of Skin* (7th ed.) New York: McGraw-Hill. 2008.
2. Lakshmi T, Radha MR, Jayshree N. Formulation and Evaluation of Herbal Gel Conatining *Dalbergia sissoo* Roxb. Bark Extract. Journal of Pharmaceutical Research & Clinical Practice, 2014;4(1):53-7.
3. Cadet, J., & Wagner, J. R. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harbor perspectives in biology*, 2013); *5*(2): a012559.
4. Zaenglein AL, Graber EM, Thiboutot DM, Strauss JS. Acne vulgaris and acneiform eruptions. Fitzpatrick's Dermatology in General Medicine. 7th ed. New York: McGraw-Hill; 2008. p. 690-703.
5. Kurokawa I, Danby FW, Ju Q, Wang X, Xiang LF, Xia L, et al. New developments in our understanding of acne pathogenesis and treatment. Exp Dermatol. 2009;18(10):821-32.
6. Zandi S, Vares B, Abdollahi H. Determination of microbial agents of acne vulgaris and *Propionibacterium acnes* antibiotic resistance in patients referred to dermatology clinics in Kerman, Iran, 2008. Jundishapur J Microbiol. 2011;4(1):17-22.
7. Nakase K, Nakaminami H, Takenaka Y, Hayashi N, Kawashima M, Noguchi N. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. J Med Microbiol. 2014;63:721-8.
8. Nishijima S, Kurokawa I, Katoh N, Watanabe K. The bacteriology of acne vulgaris and antimicrobial susceptibility of *Propionibacterium acnes* and *Staphylococcus epidermidis* isolated from acne lesions. J Dermatol. 2000;27(5):318-23.
9. Juncan, A. M. 2011. *Analysis of Some Antioxidants Used in Cosmetics by Chromatographic Methods*. Thesis. Cluj-Napoca : Babe Bolyai- University
10. Fosoyiro, S. B., Adegoke, and O.O Idowu. Characterization and partial purification of antioxidant component of ethereal fractions of Aframomum danielli. *World Chem*, 2006; 1: 15.
11. Arabshahi-Delouee S. & Urooj A. Antioxidant properties of various solvent of mulberry (*Morus indica* L.) leaves. *Food Chem*, 2007; 102 :1233–1240
12. Chen, M., *et al*. Enzimatic Hydrolisis of Corncob and Ethanol Production From Cellulosic Hydrolisate. *International Biodeterioration & Biodegradation*, 2006; 59: 85-89
13. Radojković, M. M., *et al*. Free radical scavenging activity and total phenolic and flavonoid contents of mulberry (*Morus* spp. L.,Moraceae) extracts. *Hem. Ind*, 2012; 66(4): 547–552
14. Kamiloglu, S., O. Serali, N. Unal, & E. Capanoglu. Antioxidant activity and polyphenol composition of black mulberry (*Morus nigra* L.) products. *Journal of Berry Research*, 2013; 3(1): 41–51.
15. Shon HY, Son KH, Kwon CS, Kwon GS, Kang SS. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba*, *Morus mongolica*, *Broussnetia papyrifera* vent, *Sophora flavescens* ait and *Echinosophora koreensis* Nakai. Phytomedicine. 2004;11(7-8):666-72.
16. Joshi, M., & Patravale, V. Formulation and evaluation of nanostructured lipid carrier (NLC)–based gel of Valdecoxib. *Drug development and industrial pharmacy*, 2006; *32*(8): 911-918.
17. Butkhup L, Khanprom I, Samappito S. Influence of teh Drying Process on Flavonoid Contents and Tehir Effects on Antioxidant Activity of Mulberry (*Morus*

alba L.) Green Tea Production. Thailand: Mahasarakham University; 2007.
18. Phoungchandang S, Tochip L, Srijesdaruk V. White mulberry leaf drying by tray and heat pump dehumidified dryers. World J Agric Sci. 2008;4(5):844-51.
19. Budiman, A., Aulifa, D.L., Kusuma, A.R.W., Sulastri, A., Antibacterial and Antioxidant Activity of Black Mulberry (*Morus nigra* l.) Extract for Acne Treatment. Pharmacogn J. 2017; 9(5): 611-614.
20. Budianto B, Prajitno A, Yuniarti A. Antibacterial activity Fennel fruit extract (*Foeniculum vulgare*, Mill) on *Vibrio harveyi* and *Vibrio alginolyticus* (antibacterial activity of fennel (*Foeniculum vulgare* Mill) Extract on *Vibrio alginolyticus* and *Vibrio harveyi*). J Agritech. 2017;35(3):266-72.
21. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J Pharmacol. 2005;37(1):26.
22. Budiman, A., Aulifa, D.L., Kusuma, A. S. W., Kurniawan, I.S., Sulastri, A. Peel-off gel formulation from black mulberries (*Morus nigra*) extract as anti-acne mask. National Journal of Physiology, Pharmacy and Pharmacology. 2017 ; Vol 7,Issue 9: 1-4.
23. Contreras, M. D., & Sanchez, R. Application of a factorial design to the study of the flow behavior, spreadability and transparency of a Carbopol ETD 2020 gel. Part II. *International journal of pharmaceutics*, 2002; *234*(1): 149-157.
24. Khan, M. A. Sun Protection Factor Determination Studies of Sunscreen Formulations for Their Selection and Use in Cosmetics. *Pharmaceutical Biology*, 2014; 4 (1): 9-11
25. Venkatesh, K. P. and S. Chauhan. Mulberry: Life Enhancer. *J. Med. Plants Res*. 2008; 2: 271-278
26. Minhas, M., *et al*. Evaluation of Antibiotic and Antioxidant Activity of *Morus nigra* (Black Mulberry) Extracts Against Soil Borne. *Food Borne and Clinical Human Pathogens*, 2016; 48(5): 1381–1388
27. Grajek, K., A. Wawro, and D. Kokocha. Bioactivity of *Morus Alba* L. Extracts – an Overview. *IJPSR*, 2015; 6(8)*:* 3110-3122
28. Pawlowska, A. M., W.A. Oleszek, and A. Braca. Quali-quantitative analyses of flavonoids of *Morus nigra* L. and *Morus alba* L. (Moraceae) fruits. *J. Agric. Food Chem*. 2008; 56: 3377–3380
29. Kara S. & E. A. Ercelebi. Thermal degradation kinetics of anthocyanins and visual colour of Urmu mulberry (*Morus nigra* L.). *J. Food Eng*. 2013; 116: 541–547
30. Fazaeli, M., Z. Emam-Djomeh, A. K. Ashtari, M. Omid. Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder. *Food Bioprod. Process*. 2012; 90: 667-675
31. Ozgen, M., Serce, and C. Kaya. Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits. *Sci. Hortic-Amsterdam.,* 2009; 119: 275–279.
32. Kurek, A. N. N. A., Grudniak, A. M., Kraczkiewicz-Dowjat, A., & Wolska, K. I. New antibacterial therapeutics and strategies. *Pol J Microbiol*, 2011; *60*(1): 3-12.
33. Jun M, H.Y. Fu, J. Hong, X. Wang, C. S. Yang, C.T. Ho. Comparison of Antioxidant Activities of Isoflavones From Kudzu Root (*Pueraria lobate ohwi*). *J of Food Science*. 2006; 68(6): 2117-2122.
34. McGhie, T.K., D. R. Rowan, and P. J. Edwards. Structural Identification of Two Major Anthocyanin Components of Boysenberry by NMR Spectroscopy. *J. Agric. Food Chem.* 2006; 54 (23): 8756-8761
35. Jovanović,S., O. Maćej and J. D. Djurdjević. Influence of Selected Factors on Induced Syneresis. *Journal of Agricultural Sciences*. 2004; 49(2): 205-217
36. Alfauziah, T. Q.; Budiman, A. Uji Aktivitas Antifungi Emulsi Minyak Atsiri Bunga Cengkeh terhadap Jamur Kayu (Test Activity Antifungi Essential Oil Emulsion Clove Flower on Wood Fungus). *Farmaka*, 2016, 14.1.
37. Madan, J., & Singh, R. Formulation and Evaluation of Aloevera Topical Gels. *Int.J.Ph.Sci*. 2010; 2 (2): 551-555
38. Wierenga, A. M., Lenstra, T. A., & Philipse, A. P. Aqueous dispersions of colloidal gibbsite platelets: synthesis, characterisation and intrinsic viscosity measurements. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 1998;*134*(3): 359-371.
39. Jain SK and R. Puri. Development, characterization and in vivo localization study of topical 5-fluorouracil gels: a comparative study with conventional formulation. *Curr Drug Deliv*. 2014; 11(3):401-414
40. Shahin, M., Seham, A. H., Mohammed H., Nahed, M. Novel jojoba Oil Based Emulsion Gel Formulations for Clorimazole Delivery. *AAPS PharmSciTech*. 2011; 12 (1)
41. Pokorny, J., N. Yanishlieva, and N. Gordon. *Antioxidant in Food*. England: CRC Press Cambridge. 2001.
42. Ozgen, M., Serce, and C. Kaya. Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits. *Sci. Hortic-Amsterdam.,* 2009; 119 :275–279
43. Suh, H. J., Noh, D. O., Kang, C. S., Kim, J. M., & Lee, S. W. Thermal kinetics of color degradation of mulberry fruit extract. *Molecular Nutrition & Food Research*, 2003;*47*(2): 132-135.