IJPST
Indonesian Journal of Pharmaceutical Science and Technology

Research Article
Vol. 12 No. 2 (2025): 226-233
https://doi.org/10.24198/ijpst.v12i2.54728

Antioxidant Activities of Mulberry (*Morus alba*) Root Essence in a Biocellulose Sheet Mask

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Abstract
Biocellulose (BC) is the new delivery system in cosmetic applications with advantages. Mulberry (*Morus alba*) roots have the potential to be an anti-aging agent. This research aims to determine the formulation of BC sheet mask loaded with mulberry essence extracted with NADES-UAE method and antioxidant activity. *Morus alba* root extract was formulated into three formulas of essence preparation with different concentrations of gel base Na-CMC and tested by the antioxidant activity DPPH method. BC sheet mask produced from *Acetobacter xylinum* was formulated with different concentrations of sucrose, glacial acetic acid, and ammonium sulfate. The best BC sheet mask formula was loaded with mulberry root essence and evaluated for physical characteristics. The results showed that BC formulated as a sheet mask had good physical properties: pH of 7.19; water hold capacity of 64.77%; thickness of 0.51-1.48 mm; and swelling capacity of 168%. *Morus alba* essence formula of F2 provided the best physical evaluation. Antioxidant activity testing of *Morus alba* root essence showed the inhibition of free radicals with IC_{50} value of 0.46 mg/mL. The BC-loaded NADES-UAE *Morus alba* roots essence offers potential benefits for skin health and anti-aging. This research paves the way for developing innovative and effective cosmetic formulations.

Keywords: antioxidant, biocellulose, *Morus alba*, NADES-UAE, sheet mask

Article History:
Submitted 15 May 2024
Revised 10 March 2025
Accepted 12 March 2025
Published 30 June 2025

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Citation:
Alishlah, T.; Ulfa, E.U. Antioxidant Activities of Mulberry (*Morus alba*) Root Essence in a Biocellulose Sheet Mask. Indonesian Journal of Pharmaceutical Science and Technology. 2025: 12 (2), 226-233.

Aktivitas Antioksidan Essence Akar Murbei (*Morus alba*) dalam Sheet Mask Bioselulosa

Abstrak
Bioselulosa merupakan teknologi penghantaran kosmetik baru untuk *sheet mask* dengan berbagai kelebihan. Akar *Morus alba* telah diteliti mengandung senyawa yang memiliki aktivitas antioksidan dan menjadi kandidat bahan aktif alami dalam suatu sediaan kosmetik. Penelitian ini bertujuan untuk mengetahui formulasi dan evaluasi fisik sheet mask bioselulosa yang mengandung essence ekstrak *Morus alba*, serta aktivitas antioksidannya. Serbuk akar *Morus alba* yang diekstraksi dengan metode NADES-UAE diformulasikan menjadi tiga formula sediaan essence dengan konsentrasi basis gel CMC-Na yang berbeda. *Sheet mask* bioselulosa yang dihasilkan dari *Acetobacter xylinum* diformulasikan dengan konsentrasi sukrosa, asam asetat glasial, dan amonium sulfat yang berbeda dan dilakukan evaluasi fisik. Aktivitas antioksidan sediaan diuji secara *in vitro* menggunakan metode DPPH. Hasil penelitian menunjukkan bahwa *sheet mask* bioselulosa yang diformulasikan mempunyai sifat fisik yang baik: pH 7,19; kapasitas menahan air 64,77%; ketebalan 0,51-1,48 mm; dan kapasitas pengembangan 168%. Formula essence ekstrak akar *Morus alba* F2 memberikan hasil evaluasi fisik terbaik. Uji aktivitas antioksidan sediaan dengan metode DPPH menunjukkan adanya penghambatan terhadap radikal bebas dengan nilai IC_{50} sebesar 0,46 mg/mL. *Sheet mask* bioselulosa mengandung essence ekstrak NADES-UAE akar *Morus alba* menawarkan potensi manfaat untuk kesehatan kulit dan sebagai *anti aging*. Penelitian ini membuka jalan bagi pengembangan formulasi kosmetik yang inovatif dan efektif.

Kata Kunci: antioksidan, bioselulosa, *Morus alba*, NADES-UAE, sheet mask

1. Introduction

The skin is one part of the human body with great benefits. It protects humans from various environmental influences such as free radicals from UV rays, producing oxidative stress that causes many adverse effects on the skin such as premature aging, uneven skin pigmentation, and skin cancer. *Morus alba*, the natural ingredient, has antioxidant activity to hinder free radicals.[1] *Morus alba* root containing oxyresveratrol is a promising candidate for natural active ingredients in cosmetic preparations.[2,3]

Alishlah et al. performed the application of the natural deep eutectic solvent-ultrasonic assisted extraction (NADES-UAE) method as green extraction was successful in extracting higher oxyresveratrol content from *Morus alba* roots compared to the conventional extraction method.[2] This green extraction method has advantages such as less organic solvent usage, short extraction time, and being environmentally friendly.[4] NADES-UAE extraction is efficient because evaporation is not needed like conventional methods. NADES-UAE extract results can be directly formulated into a dosage form formulation for cosmetic preparations, such as sheet masks.

Sheet mask is one of the most popular cosmetic preparations. A sheet mask is usually soaked in essence or serum that contains skincare ingredients. In skincare terms, the essence is defined as a topical dosage form that includes the plant's compounds in a highly concentrated form. Facial essence is a thin watery skincare leave-on treatment designed to help moisturize the skin preparation.[5]

Sheet masks are reported to have a good absorption and penetration profile against the skin; hence, the active compound content can provide optimal results.[6] Unfortunately, conventional sheet masks are usually made from non-woven materials produced from waste, causing environmental problems. Biocellulose (BC), a new pharmaceutical cosmetic technology, is a natural bacteria-based polymer that has attracted great interest in cosmetic applications.[7] BC sheet masks, made from coconut water and bacteria *Acetobacter xylinum*, are environmentally friendly, biodegradable, and more affordable than new technology compared to conventional sheet masks made from non-woven.[7]

Biocellulose (BC) sheet mask was formulated with different concentrations of sucrose, glacial acetic acid, and ammonium sulfate because these ingredients influence the physical characteristics of BC.[7,8] Despite many studies on mulberry and BC, the formulation of BC sheet mask loaded *Morus alba* essence extracted with NADES-UAE method and its antioxidant activity have not been examined. Hence, this research aimed to investigate the formulation and physical evaluation of BC sheet mask-loaded *Morus alba* root essence and the antioxidant activity of *Morus alba* root essence.

2. Materials and methods

2.1. Tools

The tools used in this research were water bath (Memmert, Germany), hot plate magnetic stirrer (Thermo Fisher Scientific Inc., USA), viscometer Brookfield (Brookfield Engineering Laboratories Inc., USA), Ultrasonic chamber (Thermo Fisher Scientific Inc., USA), pH meter (Mettler Toledo, Swiss), oven (Memmert, Germany), refrigerator (Toshiba, Japan), and UV-Vis Spectrophotometer Single Beam UV-1280 (Shimadzu, Japan).

2.1. Materials

Mulberry roots (*Morus alba*) were kindly provided by Rumah Sutera, Bogor, West Java, Indonesia. The roots were washed and dried in an oven at 50°C for 6 days.[2] The dried roots were ground by the grinder, then the powders were stored in an airtight container. Bacteria *Acetobacter xylinum* was purchased from the Laboratory of Microbiology, Pharmacy Faculty, Brawijaya University. The chemicals are DPPH (Sigma Aldrich, USA), and others such as sucrose, ammonium sulfate, glacial acetic acid, urea, glycerine, sodium carboxymethyl cellulose (Na-CMC), phenoxyethanol, sodium EDTA, and propylene glycol were purchased from PT. Brataco. Coconut water was purchased from a local supplier.

2.2. Methods

2.3.1. Preparation of NADES Solvent

Urea-glycerine NADES preparation is carried out using the heating method. The urea and glycerine with a 1:3 mol ratio and a certain amount of distilled water were placed in a beaker glass container and then heated at a temperature of 50°C with constant stirring at 3000 rpm for 30 minutes until a clear solution formed.[2]

2.3.2. Mulberry (*Morus alba*) Root NADES-UAE Extraction

The NADES-UAE mulberry root extract was obtained as described by Alishlah et al.[2] The extraction process was carried out with mulberry root powder:NADES solvent with a ratio of 1:20 g/ml using an ultrasonicator for 15 minutes. The mulberry root extract was centrifuged and filtered to take the supernatant. The extract is stored in a tightly closed vial and protected

from light.[2]

2.3.3. Formulation and Physical Evaluation of Mulberry Root Essence

Three essence formulas were made using the composition of the mulberry root extract essence formula (Table 1). The formula was modified from Anita et al.[9] All ingredients were weighed in specific quantities. Na-CMC was added to hot aquadest and then dispersed until homogenized. Propylene glycol was added to the Na-CMC solution and stirred until homogeneous. Phenoxyethanol and Na EDTA are each dissolved in distilled water and then added to the Na-CMC mixture. After the essence base is ready, mulberry root extract is added to the essence base and stirred until homogeneous. The remaining distilled water is added slowly while homogenizing to the total weight. Some parameter evaluations such as organoleptic, viscosity, spreadability, pH, homogeneity, and stability testing were tested.

The organoleptic evaluation was observed visually, including appearance, color, odor, and when applied to skin by tactile sensation. The viscosity was measured using a Brookfield viscometer. Spreadability was tested by the essence (500 mg) placed right in the middle of the glass, then a coverslip was put on it and left for 60 seconds, then the diameter was measured. Furthermore, the weight (50 g) was placed on the glass and allowed to stand for 60 seconds, then the diameter was measured and repeated with increasing weights of 100 g, 150 g, and 200 g. The pH evaluation was tested by 1 g of each essence diluted in 100 mL of double distilled water, and the prepared solution's pH was assessed using a pH meter. The homogeneity was tested by the essence put between the glass object and the cover glass, then pressed and observed visually for a homogeneous composition and no coarse grains. The stability testing was done by the samples placed at 4±2°C, 28±2°C, and 40±2°C, which were evaluated for physical properties for 12 weeks. Any physical changes observed during the evaluation period were recorded.[10,11]

2.3.4. Antioxidant Activity of Mulberry Root Essence

The method was modified from Alishlah et al.[12] This DPPH test uses 6 test solution concentrations of essence mulberry root in ethanol. Three cuvettes were provided: a blank (ethanol 96%), DPPH-ethanol 96%, and DPPH-test solution. After 30 minutes of dark incubation, the absorbance of each cuvette containing the solution was measured using a UV-Vis spectrophotometer with a maximum wavelength of 517 nm. The degree of color reduction of the solution indicates the radical scavenging efficiency. From the absorbance measurements obtained, the % DPPH inhibition of the test solution, and a regression curve was created. Antioxidant activity was expressed by the IC_{50} value, which was the concentration of the test solution that can inhibit the activity of DPPH free radicals by 50%. We also tested 5% vitamin C essence as a positive control. The 5% vitamin C essence was made using the same base materials and method with the chosen best formula of essence.

2.3.5. Formulation of Sheet Mask BC & Physical Characterization

All equipment must be sterilized, and ingredients were weighed as in Table 1. Fresh coconut water was filtered and put into a container. The sucrose, ammonium sulfate, and glacial acetic acid were stirred

Table 1. Formula of Mulberry Root Essence and Sheet Mask BC

No	Material of Mulberry Root Essence	F1	F2	F3
		Concentration (g)		
1	NADES Mulberry root extract	45	45	45
2	Propylene glycol	20	20	20
3	Na-CMC	0.5	1	1.5
4	Phenoxyethanol	0.7	0.7	0.7
5	Na-EDTA	0.1	0.1	0.1
6	Aquadest	33.7	33.2	32.7
No.	**Material of Sheet Mask BC**	**Formula A**		**Formula B**
		Concentration (g)		
1	*Acetobacter xylinum*	500		500
2	Coconut water	5000		5000
3	Sucrose	25		62
4	Glacial acetic acid	10		25
5	Ammonium sulfate	14		35

F1: Formula 1; F2: Formula 2; F3: Formula 3

until homogenized, then heated until boiling for 15 minutes. The 500 mL of mixture solution was poured into a tray, covered with paper, and waited until cooled. The 500 g of *Acetobacter xylinum* starter was added to the solution mixture tray and then incubated for 7-8 days at room temperature until a BC layer formed. BC was harvested, washed with running water, hand-squeezed to remove the acid content, and cleaned from the cuticula. BC was boiled in distilled water for 30 minutes, soaked in 1% hot NaOH solution for 30 minutes, rinsed again with running water, and soaked in distilled water for 1 day until the pH was neutral. Cleaned and neutralized pH BC was placed on glass plates for cutting to the size of an adult's face (diameter of 20-26 cm) with holes for the eyes, nose, and mouth. Last, the BC sheet mask was washed until clean.[13,14]

3. Result

3.1. Mulberry Root NADES-UAE Extraction

The NADES-UAE extraction process was carried out with a 1:3 NADES urea-glycerine solution in 15 minutes of ultrasonication. This extraction condition was chosen based on a previous study that gave the highest oxyresveratrol concentration in mulberry root extract.[2] The NADES-UAE extract had a brown liquid appearance. In this extraction, 5 grams of mulberry root dry powder produced 100 mL of liquid extract.

3.2. Formulation of Mulberry Root Essence

Based on the parameters of the physical evaluation of mulberry root essence, all formulas presented a good organoleptic appearance of light brown essence, as shown in Table 2 and Figure 1A. Formula 2 (F2) made with 1% Na-CMC as an essence base produced an essence with suitable consistency. Based on organoleptic, F2 was easy to apply on the skin and most well absorbed. Based on viscosity and spreadability testing, F2 meets the specification with 800 mPas and 7.4 cm, respectively (Table 2). In pH testing, all formulas showed suitable results with skin pH (4.5-8) so they do not irritate when used on the skin.[16] The three formulas also showed completely homogeneous essences. Overall, F2 had the best physical evaluation results.

Furthermore, stability testing was carried out for 12 weeks at 3 storage temperatures: 2-8 °C, 25-30 °C, and 40°C. It was stated that there were no physical changes in the three formulas of mulberry root essence compared to week 0 when it was first made (Figure 1B-D). Mulberry root essence was physically stable.

3.3. Antioxidant Activity of Mulberry Root Essence

The antioxidant activity was tested on the best formula F2 of mulberry root essence. The antioxidant activity of mulberry root essence showed an IC_{50} value of 462.[2] ppm (0.46 mg/mL) (Figure 2). As a positive control, the antioxidant activity of 5% vitamin C essence showed an IC_{50} value of 302.8 ppm (0.30 mg/mL). Our previous study showed that the extract of NADES urea glycerin (1:3) with ultrasonic for 15 minutes contains oxyresveratrol of 121.168 ppm (µg oxyresveratrol/mL extract) (ρ extract=1.09 g/mL). With a 45% concentration of extract in essence formulation, it is equivalent to 0.005% oxyresveratrol.2 It showed that a tiny amount of oxyresveratrol in the mulberry root essence gave an IC_{50} value close to 5% vitamin C as a positive control.

3.4. Formulation of Sheet Mask BC & Physical Characterization

BC obtained in Formula A did not have good organoleptic characteristics because the BC surface was not smooth and there were small holes. Meanwhile, BC obtained in Formula B had a smooth surface and no holes (Figure 3). Formula B was chosen as the best BC formula due to its better organoleptic appearance: white color, supple texture, smooth surface, and no holes.

The availability of nutrients, acidity, temperature, and oxygen largely determines the physical properties of BC. In this BC formulation, the most influential nutrients are sucrose, a key carbon source, and ammonium sulfate, a vital nitrogen source. Their optimal addition

Table 2. Physical Evaluation Results of Mulberry Essence

Parameters	F1	F2	F3	Specifications
Organoleptic	Clear light brown color, characteristic odor, easy to apply, well absorbed to skin	Clear light brown color, characteristic odor, easy to apply, well absorbed to skin	Clear light brown color, characteristic odor, easy to apply, difficult absorbed to skin	Clear light brown color, characteristic odor, easy to apply, well absorbed to skin
Viscosity (mPas)	150	800	1500	600-1000
Spreadability (cm)	11.1 ± 0.05	7.4 ± 0.08	6.1 ± 0.21	5-8
pH	6.35 ± 0.02	6.45 ± 0.08	6.55 ± 0.04	4.5-8
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous

F1: Formula 1; F2: Formula 2; F3: Formula 3

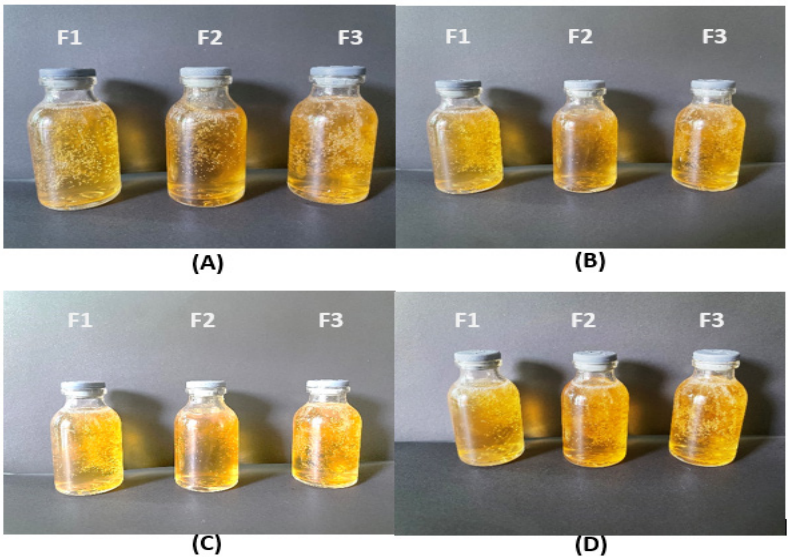

Figure 1. Essence of mulberry roots F1, F2, F3 (A) on week 0; (B) on week 12 at 2-8°C, (C) on week 12 at 25-30°C, (D) onweek 12 at 40°C (F1: Formula 1; F2: Formula 2; F3: Formula 3)

is crucial for achieving maximum BC production.[17]

Furthermore, the concentration of glacial acetic acid affects the acidity of the BC. A pH of 4.3 is considered the most suitable acidity for *Acetobacter xylinum* growth. Under alkaline conditions, the cell metabolism of these bacteria will be disrupted.[17] Hence, sufficient sucrose, ammonium sulfate, and glacial acetic acid in Formula B produced a BC with better characteristics than Formula A.

The chosen BC of Formula B was cleaned, boiled, and soaked in NaOH and distilled water until it had a neutral pH. The average pH by pH meter was 7.19 (Table 3), a suitable pH for the skin was 4.5-8.16 Neutral-pH cosmetic products will comfort the user and not irritate the skin. Then, the face-shaped cutting BC sheet masks (Figure 3C) were evaluated for physical characteristics.

The physical characterization of the BC sheet masks, on average, as seen in Table 3: weight of the BC sheet mask was 92.16 grams, the weight after 48 hours of glass pressing was 32.46 grams, and the water hold capacity was 64.77%. The thickness of the biocellulose sheet mask after being pressed was 0.51 mm. The thickness of the BC sheet mask is between 0.5-1 mm, and it is comfortable to use on facial skin.[18] BC sheet mask soaked in 30 grams of mulberry root essence. The swelling capacity showed 168%, which indicates that the essence was well absorbed by the BC sheet mask, as seen in Table 3.

4. Discussion

The extraction process with a 1:3 NADES urea-glycerine and distilled water addition could affect the viscosity and polarity of the solvent. The higher glycerine amount used in a solvent affects the increasing viscosity. Furthermore, the addition of water could adjust the properties of NADES. Water addition could also enhance solvent polarity. The use of urea-glycerine with a ratio of 1:3 with water addition (until 20 mL) could have the most similar polarity with oxyresveratrol as the target compound. Suitable viscosity and polarity of NADES solvent can improve the extraction yield significantly.[2,19]

One of the criteria of the green extraction method is to reduce energy consumption by using innovative

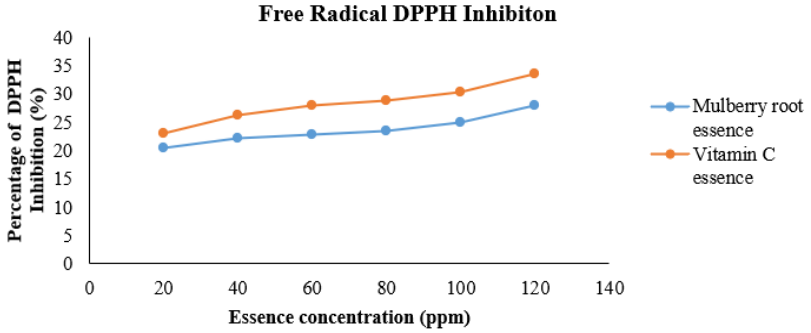

Figure 2. Percentage of DPPH Inhibition of Mulberry Root and Vitamin C Essence Curve

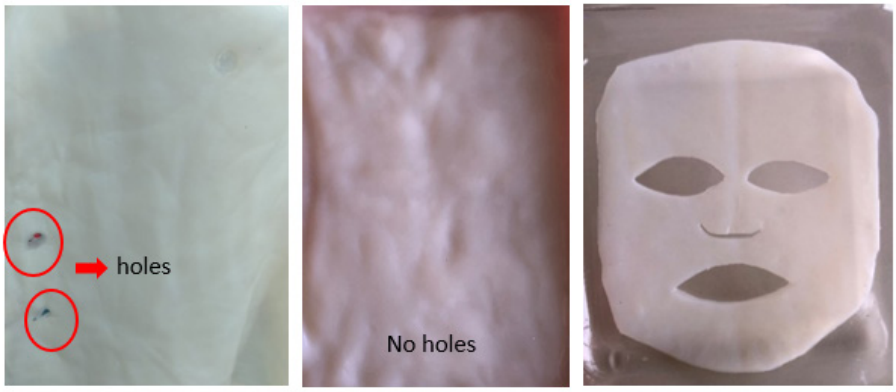

Figure 3. (a) Biocellulose of Formula A; (b) Biocellulose of Formula B; (c) Biocellulose Sheet Mask

technology such as the UAE.[20] In our previous study, the optimum extraction time using UAE to get the highest oxyresveratrol concentration was 15 minutes.

The oxyresveratrol concentration decreases when the time of extraction becomes 20 minutes.[2] The longer extraction time showed that the oxyresveratrol content in the extract was decreased, presumably due to the longer extraction time, which resulted in a higher temperature, promoting compound degradation.[21] An ultrasonic extraction time of 15 minutes could give the optimum time to allow sufficient contact time between the solvent and the sample.[2]

Alishlah et al. (2019) investigated the optimum condition (urea-glycerin 1:3 with ultrasonic time of 15 minutes) of NADES-UAE method, which produced the extract with the highest oxyresveratrol concentration (2.42 mg/g dry powder).[2] The dry powder to NADES solvent ratio was chosen to 1:20 (g/mL), therefore, the dry powder was sufficiently immersed in the solvent and well extracted.[2]

In the essence formulation, different concentrations of Na-CMC as a gelling agent influenced the viscosity, rheology, and adhesion capacity. Na-CMC is a cellulose derivative with neutral properties and can increase viscosity. The higher the Na-CMC concentration, the higher the viscosity.[22,23] The rheology type of essence is pseudoplastic, where viscosity decreases with increasing shear rate. This occurs in long-chain molecules such as polymers, including methylcellulose and carboxymethyl cellulose such as Na-CMC in this formulation.[24] The essence will stick enough to the base of the sheet mask and the skin when first used. When using a sheet mask, it will be left for a while and then removed, and we will spread the essence all over the face until it is fully absorbed into the skin. The viscosity of F2 was suitable for preparing essence because it was viscous enough on the BC sheet mask-based and easy to spread on the skin.[13]

This research showed that the mulberry root essence has antioxidant activity. It is expected to inhibit free radicals in the skin. This aligns with the previous study that determined the antioxidant activity and tyrosinase inhibition in NADES mulberry root extract with IC_{50} value of 1392.14 µg/mL and 178.43 µg/mL, respectively.[12] In B16F10 cells, *Morus alba* L. root inhibits melanin formation through sphingosine-1-phosphate (S1P) signaling by raising cellular S1P levels and suppressing S1P lyase activity. The extract from *Morus alba* roots primarily promotes MITF degradation mediated by S1P-S1PR3-ERK signaling, mitigating melanogenic effects.[25,26] Mulberry root is a potential natural agent for cosmetics such as skin brightening and anti-aging agents.[27]

Mulberry essence loaded in BC could be a new cosmetic delivery system. BC has an enormous

Table 3. Physical Characteristics of BC Sheet Mask

Sample BCsheet mask	pH	Wet Weight (gr)	After pressed weight (gr)	Water hold capacity (%)	Wet Thickness (mm)	After pressed thickness (mm)	%Swelling capacity
1	7.27	92.12	33.45	63.69	1.40	0.50	168 %
2	7.10	92.27	32.64	64.62	1.54	0.50	167 %
3	7.20	92.08	31.31	66.00	1.50	0.54	168 %
Average	7.19	92.16	32.46	64.77	1.48	0.51	168 %
SD	0.070	0.082	0.882	0.949	0.059	0.019	0.005

SD: Standard Deviation

liquid loading capacity due to its strong hydrophilic attributes. Due to its hydrophilic properties,[13,28] BC moisturizes the skin better than non-woven sheet masks.[15] *Acetobacter xylinum* bacteria manufacture cellulose and generate acid from glucose. These bacteria convert glucose and similar sources into cellulose through the pentose cycle. The long and silky cellulose derived from *Acetobacter xylinum* bacteria fibrils demonstrates strong thermal resilience.[17] Although BC shares the same molecular formula as plant cellulose ($C_6H_{10}O_5$), the BC structure does not contain lignin, hemicellulose, or pectin. BC may be synthesized with high purity using comparatively simple techniques.[29,30] BC has unique characteristics, including high mechanical strength, water-holding capacity, biocompatibility, and repeatability with the required shape and nanofiber network structure. The fibrous structure of biocellulose comprises a three-dimensional non-woven network of nanofibrils that are kept together by intra- and inter-fibrilar hydrogen bonding to form a highly strong hydrogel state.[28,31] BC may offer a bright future for cosmetic applications.

5. Conclusion

The formula of the mulberry roots essence showed F2 was the best formula with good physical properties such as organoleptic, viscosity, spreadability, pH and homogeneity. The Formula B of BC sheet mask was successfully synthesized and had the best physical characteristics: pH of 7.19, water hold capacity of 64.77%, thickness of 0.51-1.48 mm, and swelling capacity of 168%. Antioxidant activity testing of *Morus alba* root essence shows the inhibition of free radicals with IC_{50} value of 462.2 ppm (0.46 mg/mL). The BC sheet mask loaded with mulberry root essence has the potential as an antioxidant cosmetic preparation that plays a role in skin brightening and anti-aging.

Acknowledgment

This study was funded by Hibah Dosen Pemula of Jember University with contract number 3371/ UN25.3.1/LT/2023.

Conflict of Interest

The authors declare no conflicts of interest.

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