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Preparation of Readily-to-Use Stilbenoids Extract from *Morus alba* Callus Using a Natural Deep Eutectic Solvent

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Abstract: The consumer and cosmetic industries have recently placed a greater emphasis on ecofriendly solvents for botanical extraction, including natural deep eutectic solvents (NADES). In this study, NADES were prepared for *Morus alba* callus extraction. The efficiency of extraction from the NADES and methanol was investigated by comparison of the stilbenoids yield and anti-melanogenesis activity. Prior to testing the irritability of a suitable NADES on the reconstructed human epidermis (RhE), the effect of the selected NADES on stilbenoids stability was determined. The results showed that the highest yields of stilbenoids were obtained from choline chloride-glycerol mixtures (Ch1G2) and methanol extracts, with no significant difference in yields (5.06 ± 0.05 and 6.32 ± 0.40 mg/g callus dry weight, respectively). The NADES extracts of *M. alba* callus showed comparable anti-melanogenesis activity compared to methanol. In term of stability, stilbenoids in Ch1G2 remained stable after six months of storage at 4 °C except resveratrol. Furthermore, Ch1G2 had no irritation effect on RhE. Thus, based on the findings of this study, Ch1G2 is an intriguing green solvent alternative for the extraction of *M. alba* callus and may be advantageous for the preparation of skin-lightening cosmetics.

Keywords: natural deep eutectic solvent; *Morus alba*; stilbenoids; stability

Citation: Komaikul, J.; Mangmool, S.; Putalun, W.; Kitisripanya, T. Preparation of Readily-to-Use Stilbenoids Extract from *Morus alba* Callus Using a Natural Deep Eutectic Solvent. *Cosmetics* **2021**, *8*, 91. https://doi.org/10.3390/cosmetics8030091

Academic Editor: Antonio Vassallo

Received: 13 August 2021
Accepted: 18 September 2021
Published: 19 September 2021

1. Introduction

Extraction is a critical step in the process of obtaining phytochemicals from plant raw materials. Typically, an organic solvent such as ethanol has been used to extract plant raw materials for cosmetic ingredients. Following extraction, the solvent should be removed or reduced using an efficient system. Organic solvent extracts in cosmetics could cause an unsatisfactory experience for consumers and may occasionally be incompatible with other cosmetic ingredients. Besides the conventional solvent, green solvents are gaining popularity as an environmentally friendly and safe alternative that benefits both workers and consumers. Exploring green solvents for the preparation of botanical extracts is a current research trend [1].

Since 2003, deep eutectic solvents (DES) have been recognized as a novel class of sustainable solvents. DES are produced from the mixing of two or more inexpensive, biodegradable components [2]. When the DES is composed entirely of primary metabolites, such as amino acids, organic acids, sugars, or choline derivatives, it is referred to as a naturally occurring deep eutectic solvent (NADES) [3]. NADES exist around cells' membranes and are involved in the biosynthesis, solubilization, and storage of a variety of poorly water-soluble and unstable compounds in cells [4]. This generates a plethora of application possibilities for NADES. Numerous studies have demonstrated that NADES are an outstanding and promising solvent choice for sustainable and green extraction,

resulting in novel applications in the food, cosmetic, and pharmaceutical industries [5–9]. This could have the advantages of reducing the use of organic solvents and making the extract safe for use in cosmetic formulations without the need to remove the solvents [10]. Moreover, NADES are compatible with various cosmetic ingredients and may increase the extract's bioavailability [11,12].

For example, choline chloride-based NADES are commonly mixed as a hydrogen bonding acceptor for a broad spectrum of phytochemical extraction. In a previous study, the mixture of choline chloride and glycerol has been reported to be a good system for extraction of resveratrol from peanuts [13] The information from this study may further applied to the extraction of stilbenoids from other plants.

Another alternative sustainable strategy in cosmeceuticals is to use biotechnology to circumvent the limitations of natural plant sources for large-scale use. For instance, the culture conditions for *M. alba* callus were studied in order to obtain a high yield of stilbenoids in a methanolic extract with a skin lightening effect [14]. By combining this controllable source of *M. alba* with NADES extraction, a new sustainable method for producing a cosmetic ingredient might be achieved.

The purpose of this study was to determine the capacity of NADES to extract stilbenoids from *M. alba* callus and the suitability of a selected NADES for the preparation of the ready-to-use extract for skin lightening products.

2. Materials and Methods

2.1. Chemicals and Reagents

Mulberroside A, oxyresveratrol and resveratrol (>98% purity) were purchased from ChemFaces (Hubei, China). Choline chloride, sucrose, malic acid and oxalic acid were purchased from Himedia (Mumbai, India). B16-F10 (CRL-6475), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from ATCC (Manassas, VA, USA). α-Melanocyte-stimulating hormone (α-MSH), kojic acid, and synthetic melanin were purchased from Sigma-Aldrich (St. Louis, MO, USA). EpiSkin^{TM} reconstructed human epidermis (0.38 cm^3) and maintenance medium were purchased from EpiSkin (Lyon, France). All the other reagents were analytical grade.

2.2. Plant Materials

M. alba callus subcultured in Murashike & Skoog medium supplemented with thidiazuron (0.1 mg/L) and naphthaleneacetic acid (1 mg/L) [14]. After 5 weeks, the callus was collected and dried at 50 ± 5 °C for 24 h. The dried callus was ground to powder and kept in the refrigerator for further steps.

2.3. Preparation of NADES

The NADES were prepared at a specific molar ratio of choline chloride to the hydrogen bonding donor as shown in Table 1. The mixtures of choline chloride and hydrogen bonding donors at 70% *w/w* were mixed with 30% *w/w* deionized water using a magnetic stirrer at room temperature. Then, the clear solution of NADES was used as the solvent for stilbenoids extraction.

Table 1. Natural deep eutectic solvent (NADES).

Abbreviation	Hydrogen Bonding Acceptor	Hydrogen Bonding Donor	Molar Ratio
Ch1G2	Choline chloride	Glycerol	1:2
Ch1G1	Choline chloride	Glycerol	1:1
Ch1D1	Choline chloride	Dextrose	1:1
Ch1S1	Choline chloride	Sorbitol	1:1
Ch1C1	Choline chloride	Citric acid	1:1
Ch1O1	Choline chloride	Oxalic acid	1:1
Ch1M1	Choline chloride	Malic acid	1:1

2.4. Extraction

Ground dried *M. alba* callus (600 mg) was extracted with different NADES (9 mL) using ultrasound-assisted extraction (UAE) at 40 kHz for 30 min. Then, the extract was centrifuged at 9700× *g*. Finally, the clarified supernatant was transferred into a new tube which was protected from light and determined stilbenoids content using HPLC. To estimate the extraction efficiency of NADES compared with the conventional organic solvents, methanol and ethanol were used for *M. alba* callus extraction.

2.5. Stability Study

The stability study of stilbenoids in NADES was performed in the form of an extract and in the form of spiked stilbenoids in the blank solvents. The *M. alba* callus extracts were prepared as in Section 2.4 by using NADES and methanol as the solvents separately. All samples were separately kept at 4 °C, 30 °C and 40 °C. In the case of the extracts by methanol, the extracts were kept only at 4 °C. Then, the extracts were determined to stilbenoids content at 1 and 6 months after storage. Meanwhile, in the form of a spiked sample, the standard solution of mulberroside A, oxyresveratrol and resveratrol were separately prepared in methanol. Then, the standard solution was added to each solvent (Ch1G2, Ch1M1 and methanol) at a final concentration of 25 μg/mL.

2.6. Determination of Stilbenoids by HPLC Analysis

Mulberroside A, oxyresveratrol and resveratrol were used as representative stilbenoid contents in *M. alba* callus and determined by HPLC analysis. The HPLC procedure was modified from a previous study [14]. Briefly, the extract was diluted with methanol four times and filtered with a 0.22 μm syringe filter. Then, the stilbenoid contents were analyzed using an LC-10AD HPLC system (Shimadzu, Kyoto, Japan) equipped with a Cosmosil 5 C18-MS-II (5 μm, 250 mm × 4.6 mm I.D.) column and a UV detector (320 nm). The elution was performed at a flow rate of 1.0 mL/min with acidified water (1.5% acetic acid: solvent A) and acetonitrile (solvent B) with the following gradient: 6% B (0–2 min), 6–12% B (2–3 min), 12% B (3–9 min), 12–18% B (9–11 min), 18% B (11–17 min), 18–40% B (17–20 min), 40% B (20–25 min), 40–80% B (25–28 min), 80–6% B (28–32 min) and 6% B (32–44 min). The calibration curves were generated by using five concentrations of each standard compound (1.56 to 25 μg/mL). The stilbenoid contents were calculated from the peak areas.

2.7. Anti-Melanogenesis Activity

The anti-melanogenesis assay was performed on B16-F10 cells as in a previous study with some modifications [15]. The mouse melanoma cell line B16F10 (CRL-6475) was cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin solution at 37 °C in a humidified 5% CO_2 incubator. The extracts that provide a high yield of stilbenoids were selected for study in this section. Each extract was filtered with a 0.45 μm syringe filter, and then diluted with DMEM to reach a final concentration of 0.1% and 1% of the extract for testing in cell assay.

2.7.1. Cytotoxicity Assay

Cells were seeded at a density of 2500 cells per well of 96-well culture plates. After overnight incubation, cells were treated with 0.1% and 1% of the extracts or 1 mM kojic acid for 3 days with α-MSH (10 nM). Blank medium with α-MSH was used as control. Cell viability was measured using the MTT assay as previously described [16]. The percentage of cell viability was calculated.

2.7.2. Inhibitory Activity in Extracellular Melanin Production

Cells were seeded at a density of 2500 cells per well of 96-well culture plates and incubated for 24 h. The cells were treated with 0.1% and 1% of the extracts followed by stimulation with α-MSH (10 nM) for 3 days. Melanin contents in cell-free culture medium were assayed by measuring the absorbance at 405 nm using a microplate reader and were

calculated from a standard curve generated using synthetic melanin. Kojic acid (1 mM) and culture media in the absence of α-MSH were used as a positive and negative control, respectively. The percentage of melanin content was compared with negative α-MSH.

2.8. Skin Irritation Test

The skin irritation test was performed using reconstructed human epidermis (RhE) or EpiSkin^{TM} with some modifications from the OECD [17]. Briefly, the skin tissue was cultured in 2 mL of maintenance medium in a 12-well plate and incubated at 37 °C, 5% CO_2, 95%RH for 24 h. Then, 50% *w*/*v* of an extract was applied to the skin tissue and incubated for 24 h. Whereas 5% *w*/*v* sodium lauryl sulfate (SLS) and phosphate buffer saline (PBS) were used as positive and negative controls, respectively. After exposure, the skin tissue was washed with 10–15 mL of PBS 3 times and transferred to a new 12-well plate with 2 mL of maintenance medium for 42 h of incubation. The cell viability was then performed by the MTT assay. 2 mL of MTT solution (0.3 mg/mL) was added to each well. After incubation for 3 h, 500 µL of acidic isopropanol was added to dissolve the formazan from the skin tissue. The supernatant was collected and measured at 570 nm. The percentage of cell viability was calculated using Equation (1):

$$\%\text{Cell viability} = (OD_{sample}/OD_{negative\ control}) \cdot 100 \quad (1)$$

2.9. Statistical Analysis

The data analysis was performed using PASW Statistics for Windows, version 18.0 software (SPSS Inc., Chicago, IL, USA). The data was expressed as mean ± SD. The statistical significance between groups in the yield of stilbenoids and anti-melanogenesis activity derived from different solvents was determined using one-way analysis of variance (ANOVA) with Duncan's method (p-value < 0.01). The statistical significance values for the stability study were carried out using one-way ANOVA with Tukey's test (p-value < 0.01). All experiments were performed in triplicate.

3. Results

In this study, the extraction efficiency of seven different NADES was investigated by measuring stilbenoids content, including mulberroside A, oxyresveratrol and resveratrol. The extraction efficiency was compared with ethanol and methanol.

In Table 2, the extraction by Ch1G2 exhibited the highest stilbenoids content (5.06 ± 0.05 mg/g callus dry weight) among other NADES. Moreover, it was higher than the extraction yield of ethanol (0.81 ± 0.04 mg/g callus dry weight). The stilbenoids yield of stilbenoids in the Ch1G2 extract was not significantly different from that in the methanol (6.32 ± 0.40 mg/g callus dry weight).

Table 2. Extraction yield of stilbenoids in NADES (mean ± SD, n = 3).

NADES	(mg/g Callus Dry Weight)		
	Mulberroside A	**Oxyresveratrol**	**Stilbenoids Content**
Ch1G2	4.93 ± 0.04 ^{b}	0.13 ± 0.01 ^{a}	5.06 ± 0.05 ^{a,b}
Ch1G1	2.20 ± 0.04 ^{e}	n.d.	2.20 ± 0.04 ^{c,d}
Ch1D1	3.87 ± 0.16 ^{c}	n.d.	3.87 ± 0.16 ^{c,d}
Ch1S1	1.67 ± 0.11 ^{f}	0.08 ± 0.00 ^{b}	1.74 ± 0.11 ^{d}
Ch1C1	2.95 ± 0.07 ^{d}	n.d.	2.95 ± 0.07 ^{c}
Ch1O1	0.89 ± 0.04 ^{g}	0.05 ± 0.01 ^{b}	0.94 ± 0.05 ^{d}
Ch1M1	3.82 ± 0.08 ^{c}	0.13 ± 0.01 ^{a}	3.95 ± 0.08 ^{b,c}
Ethanol	0.81 ± 0.04 ^{g}	n.d.	0.81 ± 0.04 ^{d}
Methanol	6.14 ± 0.37 ^{a}	0.17 ± 0.04 ^{a}	6.32 ± 0.40 ^{a}

$^{a–g}$ indicated p-value < 0.01 in each compound; significance differences were determined using Duncan's method.; n.d. = not detected.

To determine the skin lightening properties of the *M. alba* callus extracts from NADES, anti-melanogenesis activity in B16-F10 melanoma was investigated. According to the stilbenoid yield, the extracts derived from Ch1G2, Ch1D1, Ch1M1 were chosen for the anti-melanogenesis activity investigation. By exhibiting greater than 80% cell viability, all test samples demonstrate no cytotoxicity (Figure 1). In terms of anti-melanogenesis activity, at the concentration of 1% of *M. alba* callus extracts from Ch1G2, Ch1D1, Ch1M1 and methanol revealed a significant decrease in melanin content when stimulated by α-MSH (Figure 2).

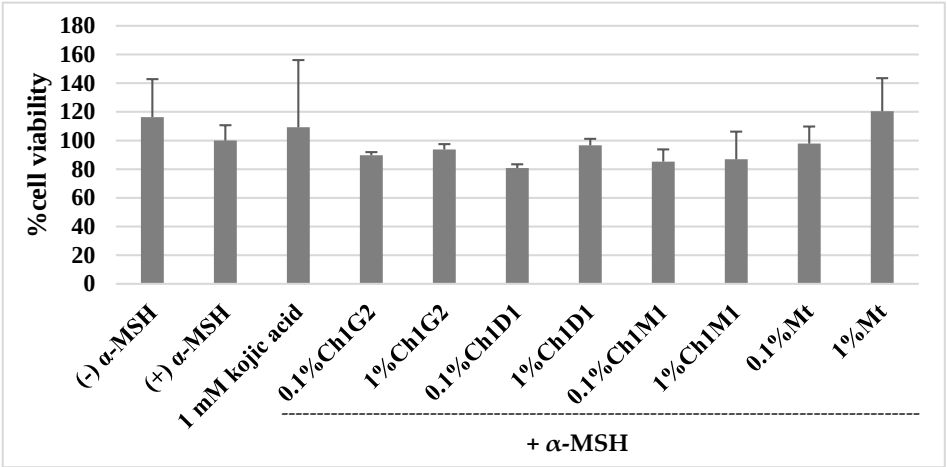

Figure 1. Percentage of cell viability of B16-F10 melanoma after treatment with kojic acid (positive control), NADES and methanol (MT) extracts of *M. alba* in the presence (+) or absence (−) of α-MSH (10 nM) (mean ± SD, $n = 3$).

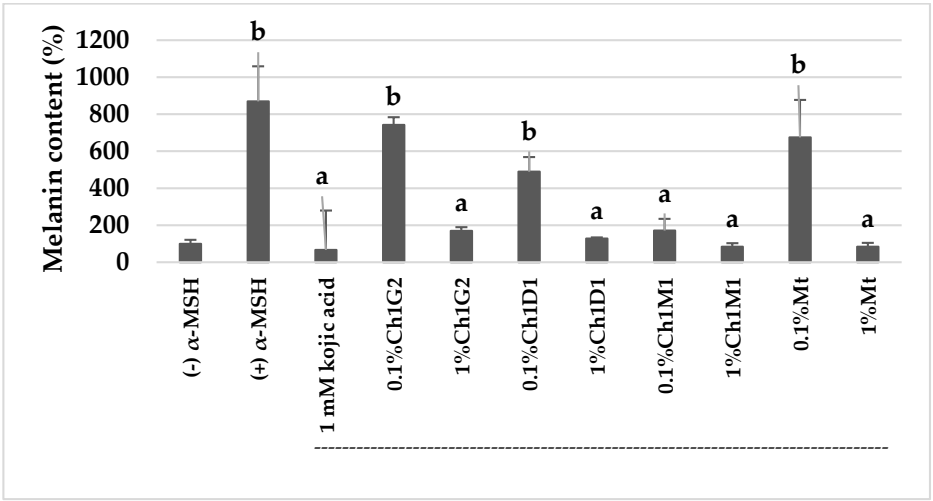

Figure 2. The percentage of extracellular melanin content from B16-F10 melanoma after treatment with kojic acid (positive control), NADES and methanol (MT) extracts of *M. alba* in the presence (+) or absence (−) of α-MSH (10 nM) (mean ± SD, $n = 3$), ^{a,b} indicated p-value < 0.01 among positive α-MSH groups; significance differences were determined using Duncan's method.

Consequently, the extracts derived from Ch1G2 and Ch1M1 were chosen to perform the stability test in comparison with the methanol extract. The extracts were stored for 1 and 6 months at 4 °C, 30 °C and 40 °C except the methanol extract, which was stored only at 4 °C due to its low flash point (9–12 °C). After storage, the remaining stilbenoids were measured by HPLC (Table 3). The results showed that mulberroside A content in the extract derived from Ch1G2 was stable for 1 month at every storage temperature and more stable after 6-month storage, in comparison with Ch1M1. The content of mulberroside A in

the extracts derived from Ch1M1 and methanol obviously decreased at 1 and 6 months. The low levels of oxyresveratrol were detected in the samples and it was not stable in all types of tested solvents except methanol at 4 °C for 1 month. Resveratrol could not be detected in the extracts.

Table 3. The stability of stilbenoids in MA extract using NADES and methanol at varying temperatures for 1 and 6 months (mean ± SD, n = 3).

Solvents		Ch1G2	Ch1M1	Methanol
4 °C		mg/g callus dry weight		
Mulberroside A (mg/g)	0 month	4.93 ± 0.04	3.82 ± 0.08	6.14 ± 0.37
	1 month	4.74 ± 0.06	2.62 ± 0.17 *	5.32 ± 0.16 *
	6 months	4.65 ± 0.09 *	2.94 ± 0.03 *	5.28 ± 0.03 *
Oxyresveratrol (mg/g)	0 month	0.13 ± 0.01	0.13 ± 0.01	0.17 ± 0.04
	1 month	0.09 ± 0.00 *	n.d.	0.11 ± 0.00
	6 months	0.05 ± 0.01 *	n.d.	0.07 ± 0.00 *
30 °C				
Mulberroside A (mg/g)	0 month	4.93 ± 0.04	3.82 ± 0.08	-
	1 month	4.75 ± 0.12	2.71 ± 0.11 *	
	6 months	2.15 ± 0.00 *	2.32 ± 0.02 *	- -
Oxyresveratrol (mg/g)	0 month	0.13 ± 0.01	0.13 ± 0.01	-
	1 month	n.d.	n.d.	
	6 months	n.d.	n.d.	- -
40 °C				
Mulberroside A (mg/g)	0 month	4.93 ± 0.04	3.82 ± 0.08	-
	1 month	5.15 ± 0.31	1.74 ± 0.07 *	
	6 months	3.18 ± 0.03 *	n.d.	- -
Oxyresveratrol (mg/g)	0 month	0.13 ± 0.01	0.13 ± 0.01	-
	1 month	n.d.	n.d.	
	6 months	n.d.	n.d.	- -

n.d. = not detected; - = not performed; * = $p < 0.01$ vs. 0 month of individual solvent, Tukey's test.

Due to the small amount of oxyresveratrol and resveratrol found in the extracts, the stability study was performed using standard spiking of oxyresveratrol, resveratrol, and mulberroside A into Ch1G2, Ch1M1 and methanol to clarify the individual stability of each stilbenoid. The study was also performed under the same temperatures and storage time conditions. In Ch1G2 and methanol, mulberroside A and oxyresveratrol content persisted more than 90% and 70%, respectively, for 6 months at 4 °C (Table 4). The mulberroside A content remained more than 90% in Ch1G2 at 40 °C. All of the spiked stilbenoids in Ch1M1 decreased in content, which was related to the increase in temperature and storage time.

To ensure the safety of leave-on products, the skin irritation test was evaluated using an extract derived from a choline chloride-based NADES. Due to the high yield and stability of stilbenoids in Ch1G2, the *M. alba* callus extract derived from Ch1G2 was selected to investigate its skin irritation effects.

The 50% *w*/*v* *M. alba* callus extract derived from Ch1G2 (MA-Ch1G2), containing approximately 15% *w*/*v* of choline chloride, was applied on a reconstructed human epidermis model. The skin irritation test results were expressed as a percentage of cell viability compared with PBS and 5% *w*/*v* SLS as negative and positive controls, respectively. In Figure 3, the percentage of cell viability of MA-Ch1G2 was 82.36% which is categorized as safe according to the OECD guideline. Thus, 50% *w*/*v* MA-Ch1G2 or its lower concentration can be readily applied in cosmetic formulations without skin irritation effect.

Table 4. The stability of added stilbenoids in NADES and methanol at varying temperatures for 1 and 6 months (mean ± SD, *n* = 3).

DES		Ch1G2	Ch1M1	Methanol
4 °C		μg/mL (Percentage of residue content)		
Mulberroside A	1 month	24.80 ± 0.48 (99.20%)	20.20 ± 0.96 (80.80%)	24.92 ± 1.80 (99.68%)
	6 months	23.08 ± 0.80 (92.32%)	16.88 ± 0.40 (67.52%)	24.24 ± 1.08 (96.96%)
Oxyresveratrol (μg/mL)	1 month	17.52 ± 0.24 (70.08%)	15.80 ± 0.52 (63.20%)	19.68 ± 0.92 (78.72%)
	6 months	17.56 ± 0.64 (70.24%)	13.12 ± 0.32 (52.48%)	17.84 ± 0.40 (71.36%)
Resveratrol (μg/mL)	1 month	16.08 ± 0.44 (64.2%)	16.00 ± 1.52 (64.00%)	23.40 ± 1.48 (93.60%)
	6 months	17.96 ± 0.72 (71.84%)	13.88 ± 0.52 (55.52%)	20.00 ± 1.60 (80.00%)
30 °C				
Mulberroside A (μg/mL)	1 month	20.12 ± 0.24 (80.48%)	13.76 ± 0.88 (55.04%)	-
	6 months	22.24 ± 0.48 (88.96%)	10.76 ± 0.52 (43.04%)	-
Oxyresveratrol (μg/mL)	1 month	15.04 ± 0.28 (60.16%)	13.32 ± 0.44 (53.28%)	-
	6 months	7.68 ± 0.08 (30.72%)	3.36 ± 0.04 (13.44%)	-
Resveratrol (μg/mL)	1 month	15.80 ± 1.20 (63.20%)	14.08 ± 0.88 (56.32%)	-
	6 months	14.84 ± 0.60 (59.36%)	9.80 ± 0.08 (39.20%)	-
40 °C				
Mulberroside A (μg/mL)	1 month	23.68 ± 1.28 (94.72%)	8.96 ± 0.44 (35.84%)	-
	6 months	23.56 ± 0.28 (94.24%)	n.d. -	-
Oxyresveratrol (μg/mL)	1 month	17.08 ± 0.76 (68.32%)	9.80 ± 0.16 (39.20%)	-
	6 months	n.d. -	n.d. -	-
Resveratrol (μg/mL)	1 month	17.28 ± 1.00 (69.12%)	12.56 ± 1.04 (50.24%)	-
	6 months	5.76 ± 0.12 (23.04%)	n.d. -	-

n.d. = not detected; - = not performed.

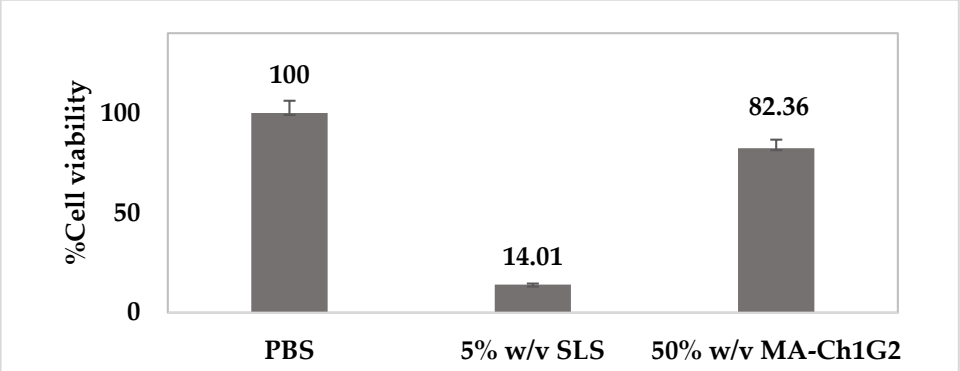

Figure 3. The percentage of cell viability after treatment with 50% *w/v M. alba* callus extracts from Ch1G2 (MA-Ch1G2), PBS and 5% *w/v* SLS as negative and positive controls, respectively. (mean ± SD, $n = 3$).

4. Discussion

4.1. Capacity of NADES for M. alba Callus Extraction

NADES have been used to increase the solubility and stability of compounds that are hydrophobic and to conduct novel sustainable and green extractions. Combining NADES and biotechnology enables the production of a unique active ingredient for cosmetics while maintaining an economically viable and sustainable process [11,12].

To achieve an effective extraction method, popular techniques such as microwave-assisted and ultrasound-assisted extraction have been frequently used in combination with NADES [8,11]. However, our preliminary study found that microwave-assisted extraction altered the conformation of stilbenoids in the *M. alba* callus extract, causing a change to their less effective *cis*-forms (data not shown). As a result, this study employed ultrasound-assisted extraction.

The most intriguing result in our study came from the extraction with a mixture of choline chloride and glycerol (Ch1G2), which extracted stilbenoids with a yield comparable to that of methanol extraction and acceptable stilbenoid stability. Similarly, in earlier reports choline chloride-based NADES exhibited efficiency in extracting phenolic compounds [18,19]. According to the results of a skin irritation test, Ch1G2 is safe for leave-on cosmetics. In further study, the compatibility of NADES in cosmetic formulations is required to complete the overall information.

4.2. Stilbenoids Content and Anti-Melanogenesis Activity

Previously, it was suggested that urea-glycerol-based NADES was a viable option for extracting oxyresveratrol from *M. alba* roots [20]. However, to obtain a variety of active compounds with anti-melanogenesis activity from *M. alba* callus, this study used a choline chloride-glycerol-based NADES.

The Ch1G2 extract of *M. alba* callus contained a high stilbenoid yield and exhibited good anti-melanogenesis activity. The stilbenoid content is probably a good bioactivity marker for *M. alba* callus extracts. However, those of the Ch1M1 extract were contrary. The extraction with Ch1M1 provided a low stilbenoid content in comparison with methanol (Table 2), while the 0.1% of Ch1M1 extract showed the highest anti-melanogenesis activity compared with the same concentration of other solvents. This could be due to synergistic effects of non-stilbenoid compounds, including malic acid within the solvent. Malic acid is one of the α-hydroxy acids (AHAs) which have been reported to be effective in treating hyperpigmentation. The underlying mechanism of the anti-melanogenesis activity of malic acid is still not clear. Some AHAs, such as glycolic acid and lactic acid, have been reported to suppress melanin formation by directly inhibiting tyrosinase activity, which might be possible in malic acid [21,22]. Although Ch1M1 is not suitable for the extraction of *M. alba* stilbenoids, it could have potential to extract other non-stilbenoid compounds with

anti-melanogenesis properties. Our findings corroborate previous reports that NADES can be optimized to extract compounds with a range of polarity degrees [23].

The capacity of a mixture of choline chloride and glycerol to extract stilbenoids in this study is consistent with a previous report on stilbenoids extraction in peanuts [13]. The information may be further applied to the extraction of stilbenoids from other plants, such as *Artocarpus lacucha*. However, due to the small amount of resveratrol in *M. alba* callus, it was not detected from the extracts in all types of solvent in this study. In an earlier study, *M. alba* callus was reported to contain only a small amount of resveratrol, which required elicitation or post-harvest treatment to increase [14]. In nature, the roots of *M. alba* also show a variation in stilbenoids content in different cultivation regions [24]. Besides that, stilbenoids content might depend on the cultivation batch, variation in culture or harvesting process [25].

4.3. Stilbenoids Stability

The stilbenoids stability in the selected NADES was clarified by stilbenoids spiking. Although the spiked resveratrol content in methanol was more than 90% stable at 4 °C for 1 month, the flammability and toxicity of methanol limit the direct application of the extract in cosmetic formulations [26]. Consequently, conventional organic solvents must be eliminated and the limit of solvent residues ensured as indicated by the pharmaceutical or cosmetic products regulations [27]. Therefore, Ch1G2 could be an alternative instead, which should be stored at 4 °C to preserve the stilbenoid content (70–90%) for six months.

The extract prepared using the optimal NADES demonstrates its potential as a ready-to-use extract. However, because cosmetics should be stable for at least two years, additional development would be beneficial. To increase the stability of stilbenoids, stabilizers such as antioxidants and photoprotective agents might be added to the extract or finished products [28]. Additionally, to modify the stilbenoid extract, stabilization techniques such as nanocarriers may be used [29]. These could be interesting and branching strategies for synergistically increasing the stability and bioactive efficiency of stilbenoids to establish novel products.

5. Conclusions

This study demonstrated the extraction yields and the variable stability of stilbenoids in NADES extracts. The capacity of Ch1G2 to extract stilbenoids from *M. alba* callus was determined to be superior to that of six other NADES and comparable to that of methanol. Additionally, the Ch1G2 extract of *M. alba* callus is non-irritating. The extraction capacity and safety of the Ch1G2 extract indicated that Ch1G2 could be preferable for cosmetic use. Combining NADES extraction with in vitro cultivation of *M. alba* exemplified that sustainable extraction could be achievable.

Author Contributions: Conceptualization, T.K. and W.P.; methodology, T.K. and J.K.; software, T.K.; validation, T.K.; formal analysis, T.K. and J.K.; investigation, T.K. and J.K.; resources, W.P., J.K., T.K. and S.M.; data curation, T.K. and W.P.; writing—original draft preparation, T.K. and J.K.; writing—review and editing, T.K., W.P. and J.K.; visualization, T.K.; supervision, W.P. and S.M.; project administration, T.K.; funding acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Thailand Research Fund and Office of the Higher Education Commission, grant number MRG6280161.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was conducted and supported by Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Conflicts of Interest: The authors declare no conflict of interest.

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