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ABSTRACT

This study aimed to verify the potential of maqui berry extract as natural product in the pigment of semi-permanent cosmetics. ICP-MS and ICP-OES were performed for analysing heavy metal and potassium contents, respectively. The pigskin-adherence capacity was measured to identify skin-adherence stability; the cytotoxicity was tested via the MTT assay, and the dispersibility and stability of the pigment were analysed. The maqui berry extract mixtures insignificantly varied in L*, a*, and b* from the stock solution for samples with up to 50% maqui berry extract. The pigskin to which the 10-50% maqui berry extract-mixed samples were applied exhibited similar L* and a* to the pigment stock solution, but exhibited lower b*. The ICP-MS result showed that the sample containing 50% maqui berry extract had more than 50% reduced levels of heavy metals (Cd, Cr, As, Pb, and V). In optical microscopy, the dispersed state was stable for the samples with maqui berry extract 70%, whereas those containing 90% extract had an unstable dispersion state. The MTT assay showed 80% or higher cell viability of up to 5 µg/mL. After using maqui berry extract-mixed samples, the pigskin insignificantly varied in terms of L*, a* and b* from those of the pigment stock solution.

Keywords: Semi-permanent makeup, Maqui berry, Heavy metal, Pigment, Antioxidant capacity, Natural extracts.

1. INTRODUCTION

Makeup can be quickly applied using semi-permanent cosmetics, ensuring convenience, utility, and aesthetics to customers [1]. Further, there is an increased emphasis on creating a favourable impression, as well as pressure to follow trends popular in social networking culture, as people increasingly share their daily life online. As a result, the demand for semi-permanent cosmetics is rapidly increasing [2]. In addition, the obligation to wear masks, as per national policy, in response to the persistent COVID-19 (coronavirus disease) pandemic has further increased the interest in semi-permanent cosmetics than that in non-permanent cosmetics [3].

Semi-permanent cosmetics is a technique of injecting micro-pigments between the basal and granular layers of the skin epidermis. The basic principle is the natural discoloration by the desquamation of cells through skin turnover [2]. In contrast to tattooing, where pigments are permanently injected into the dermal layer [4], the injection of pigments reaches only up to the granular layer of the epidermis in semi-permanent cosmetics such that the retention time, for example, in the case of eyebrows is one to three years and not permanent [5]. Thus, convenience and natural retention of semi-permanent cosmetics has attracted a large population of consumers [2]. Nevertheless, pigment blurring, fading, and the variations in light and shade have induced consumer dissatisfaction [6]. In particular, heavy metals (Cd, Cr, As, Pb, and V) in the pigment dye have high probability of causing skin-related issues such as skin cancer [7]. The procedure-related after-effects reportedly include allergic reactions due to incorrect procedures and keloid-scar formation caused by the overproliferation of collagenous fibres [6]. Hence, expert personnel and the stability of the dye are critical in semi-permanent cosmetic procedures [8]. However, an in-depth analysis of the pigmentation that contributes to the long-lasting effect of semi-permanent cosmetics or an ink-composition analysis of heavy metals as per the expert personnel, ink quality, and pigment injection depth in the skin are lacking [6].

The Ministry of Environment classified cosmetic tattoo dyes as a *product with risk concern* as per the Registration and Evaluation of Chemical Substances Act on 26 June 2015, which was followed by the announcement of an implementation of safety criteria on 26 September 2015. This prohibits the use of such products or limits their content [7]. The 2016 survey of the current status of safety conducted by the Korea Consumer Agency revealed that heavy metals above the safety criteria were detected in 12 out of the 25 commercial semi-permanent cosmetic tattoo dyes (48.0%) [8]. Hence, a complementary measure is required because using such dyes and pigments can increase the risk of exposure to hazardous chemical substances [7]. Previous studies on pigments in semi-permanent cosmetics include a heavy metal analysis on the dyes and pigments used in cosmetic tattoos [7], composition analysis of the brown-ink pigment used in semi-permanent cosmetics [6], sensory analysis of the semi-permanent eyebrow product in varying pH [9], and water-base pigmentation analysis according to the treatment after applying semi-permanent cosmetics [2]; the scope is limited pigments in semi-permanent cosmetics.

Compared to many other natural extracts, maqui berry extract has been reported to exhibit outstanding antioxidant, antibacterial, anti-inflammation, and anti-ageing effects with high contents of polyphenols, flavonoids, anthocyanins, pigments, organic acids, and tannins [10]. Thus, this study aimed to verify the potential application of maqui berry in semi-permanent cosmetics, by reducing the hazardous components such as heavy metals in the pigments to enhance the safety of such products. This study investigated the feasibility of semi-permanent cosmetic pigment mixed with maqui berry extracts as a natural product, for which mixtures of semi-permanent pigment and natural maqui berry extracts were fabricated in varying ratios, and the pigment dispersibility and stability were assed.

2. MATERIALS AND METHODS

Materials

Freeze-dried maqui berry powder (imported from Chile by Sungpoong Co., Ltd., Anseong-si, South Korea) was used in this study. For the semi-permanent cosmetic pigment, product No. 12 (P2, Gimhae-si, South Korea) was purchased.

Maqui berry extraction

To prepare maqui berry extracts, 200 g of maqui berry powder and 1 L of 80% ethanol were mixed, sealed, and kept at room temperature (15°C–20°C) for 24 h for immersion extraction. The extracted solution was then placed in a water bath for vacuum evaporation at 40 °C using a rotary vacuum evaporator (EYELA, N-1300, Shanghai Eyela Co., China). The enriched product was stored at 4 °C.

Antioxidant activity of maqui berry extract

For the reference DPPH (2,2-diphenyl-1-picrylhydrazyl) sample, DPPH powder (free radical; 95%) purchased from Alfa Aesar was used. For the analysis, maqui berry extract was diluted in ethanol at 0, 0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL, which was added to 0.12 mM DPPH solution prepared in methanol. The control group was ascorbic acid; 100 µL extract of each concentration (0, 5, 10, 12.5, 25, and 50 µL/mL) was mixed with 900 µL of DPPH solution, and the mixture was left to react in a dark room for 30 min. After the reaction, the absorbance was measured at 517 nm by UV-Vis(Ultraviolet-visible spectroscopy). The inhibition rate (IR) for the DPPH-scavenging activity was calculated using the following equation:

$$\text{Inhibition}\% = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

$$(\text{Abs}_{\text{sample}} = \text{Abs}_{\text{test}} - \text{Abs}_{\text{colour}})$$

Antibacterial activity of maqui berry extract

Bacterial strains of *S. aureus* (NA medium, 36 °C), *P. aeruginosa* (NA medium, 36 °C), *E. coli* (NA medium, 36 °C), *C. albicans* (PDA medium, 30 °C), *S. epidermidis* (NA medium, 36 °C), and *P. acnes* (TSA medium, 36 °C) were cultured and used for measuring the antibacterial activity of the maqui berry extract. In the preculture, the bacterial strain stored in the cell stock was spread on a solid medium and placed in an incubator for 18 h. Subsequently, 3 mL of a suitable liquid medium for each strain was placed in a test tube. One colony from each cultured bacteria was collected, inoculated in a test tube, and subcultured for 18 h in an incubator before the main experiment. A paper disc (9 mm) for antibacterial activity was used, with the samples being treated at 40

μL. Next, 100 μL of each strain subcultured in the liquid medium was spread on a suitable solid medium, and the paper disc with 40 μL of absorbed extract was used for a 24-h culture in the incubator at 37 °C. The clear zones (mm) around the paper disc were then counted to comparatively analyse the antibacterial activity.

Cytotoxicity assay of maqui berry extract

To perform the cytotoxicity assay, the cells of the CCD-986SK (ATCCC CRT-1947) cell line were suspended in a freezing medium, and 1 mL of the extract was aliquoted. After freeze-drying, the mixture was transferred into a liquid nitrogen container for storage. The culture condition was a 5% CO₂ incubator at (37 ± 1) °C. The test material was diluted to five concentration levels (0.01, 0.1, 0.5, 1, and 5 μg/mL). The cells were aliquoted in a 96-well plate at 1 × 10⁴ (200 μL/well) and cultured for 24 h in a 37 °C, 5% CO₂ incubator. After the replacement with serum-free medium containing varying concentrations of the test material, the cells were cultured for 24 h in a 37 °C, 5% CO₂ incubator. The MTT (3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide) solution was aliquoted at 200 μL/well for a 2-h culture in a 37 °C, 5% CO₂ incubator. Next, the medium was removed, and the DMSO (dimethyl sulfoxide) solution was applied at 150 μL/well. The extraction proceeded for 20 min on a plate shaker, and the absorbance was measured at 540 nm through ELISA. The cell viability of the maqui berry extract was calculated using the equation below.

$$\text{Cell Viability}\% = \frac{\text{Sample solution OD} - \text{Negative Control OD}}{\text{Dx100}}$$

Inhibition of intracellular collagenase activity by maqui berry extract

To assess the collagenase activity of maqui berry extract, the test material was diluted to 0.5, 1, and 5 μg/mL, aliquoted in a 60-mm plate at 1 × 10⁴ (5 mL/plate), and cultured for 24 h in a 37 °C, 5% CO₂ incubator. The medium was removed, and the cells were washed with phosphate buffered saline (PBS). After adding 2 mL of PBS, irradiation by 10 mJ of UVB (312 nm) was performed. Following the replacement with serum-free medium containing the test material and positive control, the cells were cultured for 24 h in a 37 °C, 5% CO₂ incubator. The BCA (bicinchoninic acid) protein assay reagent kit (Pierce, Thermo Scientific, USA) was applied to the retrieved cell-culture solution, and the protein content was measured using the BSA (bovine serum albumin) standard curve. Subsequently, the Human MMP-1 ELISA Kit (Sigma-Aldrich, Missouri, USA) was applied to the retrieved cell-culture solution, and the collagenase content was measured using the respective standard curve. The intracellular collagenase content was then converted to the collagenase content by the protein content using the following equation:

$$\text{Intracellular collagenase content \%} = \frac{\text{sample group collagenase}}{\text{protein in non-sample group (collagenase/protein, Control)}} \times 100$$

Preparation of maqui berry extract-mixed samples

To prepare the maqui berry extract-mixed samples, semi-permanent cosmetic pigment No. 12 was used, and the pH and conductivity were set to 5.33 and 22.67 μs/cm. The pH and conductivity of the 80% maqui berry immersion extract was 3.95 and 206 μs/cm, respectively. The stock solution of the semi-permanent cosmetic pigment (M0 (0%)), the five different maqui berry extract mixtures (M1 (10%), M2 (25%), M3 (50%), M4 (70%), and M5 (90%)), and the solution of the maqui berry extract (M6 (100%)) were prepared in each respective mixing, ratio as shown in Table 1. Samples M1-M5 were prepared by dispersion for 30 min at 100 rpm for 30 min. The concentration ratio of each maqui berry extract mixtures is shown in Table 1.

Table 1. Concentration of maqui berry extract-mixed samples

No.	Ratio (%)	Pigment (g)	Maqui berry extract (g)	Total (g)
M0	0	2.50	0.00	2.50
M1	10	2.25	0.25	2.50
M2	25	1.88	0.62	2.50

3	M	50	1.25	1.25	2.50
4	M	70	0.75	1.75	2.50
5	M	90	0.25	2.25	2.50
6	M	100	0.00	2.50	2.50

Colorimetric analysis of maqui berry extract-mixed

To measure the colour change in the pigments of maqui berry extract-mixed samples, a 3R needle cartridge (Dongbang Medical, Seongnam, Korea) was applied to the digital machine used in semi-permanent cosmetic procedures (CF, Co., Ltd., Seoul, Korea; rated voltage AC 100-240 V, 50/60 Hz, 0.3A). With the injection of colour, the M0-M6 samples on a rubber board (semi-permanent rubber board, BM, Pyeongtaek, Korea) and the pigskin (Micro pig FCM, APURES Co., Ltd, Korea) were examined through colorimetry (CR-400, Konica Minolta, Tokyo, Japan) for comparative analysis. The mean of the triplicate measurements was obtained to maximise the accuracy. The measured values were expressed as L* (lightness), a* (redness), and b* (yellowness), and the colour difference from the stock solution of semi-permanent cosmetic pigment was analysed based on ΔE^*_{ab} calculated using the equation below.

$$\Delta E^*_{ab} = L^*2 - L^*12 + a^*2 - a^*12 + b^*2 - b^*12$$

ΔL^* (L* sample - L* standard) = difference in lightness and darkness (+ = lighter, - =darker)

Δa^* (L* sample - a* standard) = difference in red and green (+ = redder, - =greener)

Δb^* (L* sample - b* standard) = difference in yellow and blue (+ = yellower, - =bluer)

Colour change in maqui berry extract-mixed samples over time

Samples M0-M6 were applied to a rubber board at 50 °C to measure the colour change in the maqui berry extract mixtures over time. The colour after one, two, and three months was measured by colorimetry, and the mean of the triplicate measurements was obtained to maximise the accuracy. The measured values were expressed as L* (lightness), a* (redness), and b* (yellowness), and the colour difference from the stock solution of the semi-permanent cosmetic pigment was analysed based on ΔE^*_{ab} .

Heavy metal and mineral contents in maqui berry extract-mixed samples

ICP-MS (inductively coupled plasma mass spectroscopy, Agilent, 7900, USA) and ICP-OES (inductively coupled plasma-optical emission spectrometry, Agilent, 5100, USA) analyses were performed to determine the contents of heavy metals and minerals. First, microwave digestion was performed as pretreatment to dissolve the heavy metals. For this step, 0.2 g of sample, 6 mL of HNO₃, and 1 mL of H₂O₂ were mixed, with the final volume up to 50 mL. Next, the digested products at minimum amounts were concentrated via volatilisation, and then diluted with water to prepare the test solution; the nitrogen concentration of the final test solution was set to 10% or below. The standard calibration curve was plotted using the ICP standard solution, and 10 g of the pretreated sample was applied after checking the linearity ($r \geq 0.999$). For As, Cd, Cr, Pb, and V that are categorised as heavy metals and predicted to be at a relatively low concentration, ICP-MS was used to allow more precise ppt or ppb unit analyses of samples with a concentration of 0.1 ppm or below. In addition, CP-OED was used for determining the content of minerals such as K.

Dispersion stability of maqui berry extract-mixed samples

For testing the stability and dispersibility of maqui-berry-mixed extracts, optical microscopy (OM) was performed at 100× and 400× for samples M0-M6.

Cytotoxicity assay on maqui berry extract-mixed samples

To test the cytotoxicity of maqui berry extract mixtures, 50% maqui berry extract-mixed (M3) sample was applied to the cells of the CCD-986SK (ATCC CRT-1947) cell line suspended in a freezing medium. Subsequently, 1 mL of this solution was aliquoted, freeze-dried, and transferred into a liquid nitrogen container for storage. The culture condition was a 5% CO₂ incubator at (37 ± 1) °C. The test material was diluted to five concentration levels (0.01, 0.1, 0.5, 1, and 5 µg/mL). The cells were aliquoted in a 96-well plate at 1 × 10⁴ (200 µL/well) and cultured for 24 h in a 37 °C, 5% CO₂ incubator. Following the replacement with serum-free medium containing varying concentrations of the test material, the cells were cultured for 24 h in a 37 °C, 5% CO₂ incubator. After washing two times with PBS, the MTT solution (5 mg/mL, serum-free medium) was aliquoted at 200 µL/well and cultured for 2 h in a 37 °C, 5% CO₂ incubator. The medium was removed at the end of incubation, and the DMSO solution was applied at 200 µL/well. The extraction proceeded for 20 min on a plate shaker, and the absorbance was measured at 570 nm through ELISA. The cell viability for maqui berry extract-mixed samples was calculated using the following equation:

$$\text{Cell Viability\%} = \frac{\text{Sample solution O.D.}}{\text{Negative Control O.D.}} \times 100$$

Pigskin pigment-adherence test on maqui berry extract-mixed samples

For testing the pigskin pigment-adherence capacity of the maqui berry extract-mixed samples, the colour change in the pigskin for samples M0-M6 was measured through colorimetry for comparative analysis. To determine the level of pigment adherence, a 3R needle cartridge was applied to the digital machine used in semi-permanent cosmetic procedures, and the maqui berry extract-mixed samples at different concentrations were injected into the pigskin for 1 min at 1,300 rpm. A cotton ball with foam cleanser was applied to rub it with a finger at constant pressure 10 times each, followed by a cotton ball soaked in distilled water 10 times each, during which time the colorimetry measurements were conducted. The mean of the triplicate measurements was obtained to maximise the accuracy. The colour difference from the stock solution of the semi-permanent cosmetic pigment was analysed based on ΔE^*_{ab} .

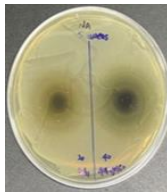
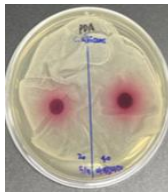
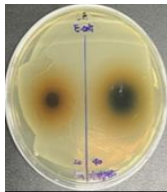
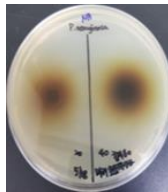
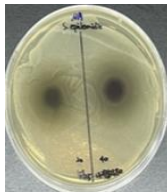
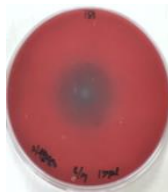
3. RESULTS AND DISCUSSION

Antioxidant activity of maqui berry extract

Maqui berry extract reportedly exhibits an outstanding antioxidant activity and can be used as a new and functional material in foods and other products as an antioxidant [14, 15]. The antioxidant activity of the maqui berry immersion extract revealed that the IC₅₀ of maqui berry extract (immersed in 80% ethanol) was 2.55, indicating good antioxidant activity, compared to the IC₅₀ of the control ascorbic acid at 1.86. Although the effects were slightly lower than those of ascorbic acid, the maqui berry extract exhibited a high level of antioxidant capacity in a concentration-dependent manner. Figure 1 illustrates the graph comparing the antioxidant activities of the maqui berry extract and ascorbic acid.

Antibacterial activity of maqui berry extract

The antibacterial activity test of the maqui berry extract revealed a high level of antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. epidermidis*, and *P. acnes*, with clear zones of 30 mm or above (Figure 2); however, this was not the case against *C. albicans*.

	<i>S. aureus</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>P. acnes</i>
Paper disc						
Clear zone (mm)	40 µL-36	ND	40 µL-33.5	40 µL-30	40 µL-32	40 µL-29

Cytotoxicity assay of maqui berry extract

The MTT assay of maqui berry extract showed cell viability of 95.9 ± 4.40 , 99.6 ± 2.70 , 99.2 ± 3.20 , 99.7 ± 2.40 , and $104 \pm 4.50\%$ for the cells treated with the extract at 0.01, 0.1, 0.5, 1, and 5 $\mu\text{g/mL}$, respectively. This result indicated that the maqui berry extract exhibited 90% or higher cell viability at a concentration of up to 5 $\mu\text{g/mL}$. Figure 3 illustrates the MTT assay result.

Inhibition of intracellular collagenase activity by maqui berry extract

The assessment of collagenase inhibition by maqui berry extract showed that the inhibition rate was 98.4 ± 3.80 , 88.7 ± 2.10 , and $86.1 \pm 4.00\%$ at 0.5, 1, and 5 $\mu\text{g/mL}$ of maqui berry extract, respectively. The highest rate of $86.1 \pm 4.00\%$ was observed in the test material treated with 5 $\mu\text{g/mL}$ of maqui berry extract. In addition, compared to the control, treatment with 100 $\mu\text{g/mL}$ of adenosine (positive control) led to $81.1 \pm 1.60\%$ inhibition of intracellular collagenase activity, indicating that the maqui berry extract can exhibit an anti-wrinkle effect. Figure 4 shows the effect of the maqui berry extract on intracellular collagenase inhibition.

Colorimetric analysis on maqui berry extract-mixed samples

Figure 5 shows the result of colour analysis conducted for each ratio of maqui berry extract-mixed samples. No significant difference was observed in the appearance for samples M0-M4. Notably, while the samples on the rubber board did not show a significant difference up to sample M5, a purplish hue was observed on the pigskin for sample M5. The colorimetry results shown in Figures 6 and 7 indicated that the L^* , a^* , and b^* values were similar for M1-M3, whereas the M0 stock solution showed no significant difference. In addition, L^* and a^* for the pigskin applied with M0-M3 samples did not vary significantly, although a decreasing trend in b^* was observed (Figures 8 and 9). This is presumed to be due to the - value of b^* measured for 100% maqui berry extract, with a higher level of added extract leading to lower b^* value.

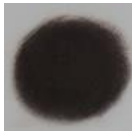

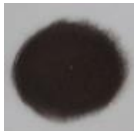

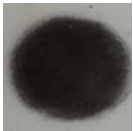
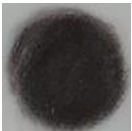
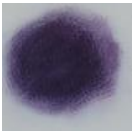







Sampl es	M0	M1	M2	M3	M4	M5	M6
Rubber board							
Micro pig							

Figure 5. A comparison of adherence capacity exhibited by ratio of maqui berry extract-mixed samples

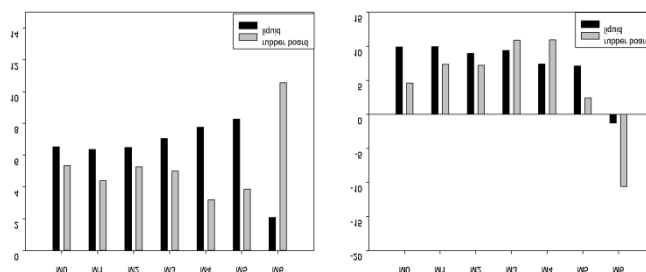


Figure 6. Result of colour difference analysis of maqui berry extract-mixed samples

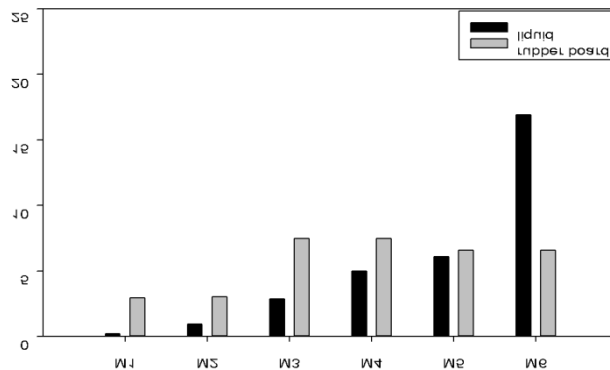


Figure 7. ΔE^*_{ab} analysis results of maqui berry extract

The analysis of ΔE^*_{ab} against the stock solution of the semi-permanent cosmetic pigment revealed that the values for M1-M6 were 0.21, 0.95, 2.87, 4.98, 6.07, and 16.8, respectively (Figure 7). Thus, the values 10.0 or above were obtained for the 100% maqui berry extract and 6.0 or below for M1-M5. However, the values were uniformly 10.0 or below for M1-M6 on the rubber board, which suggests its potential use as a pigment in semi-permanent cosmetics.

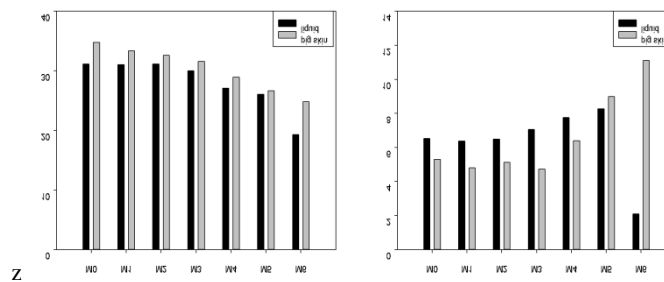


Figure 8. Results of the colorimetric analysis of the pigskin applied with maqui berry extract-mixed samples

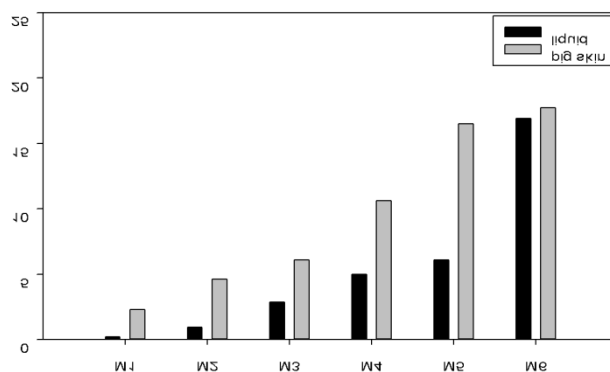


Figure 9. ΔE^*_{ab} analysis result for pigskin with maqui berry extract

ΔE^*_{ab} analysis of the maqui berry extract-mixed samples on the surface of the pigskin showed that the values for M1-M6 were 2.31, 4.62, 6.07, 10.6, 16.5, and 17.7, respectively (Figure 9), indicating 10.0 or below. Thus, the stability of the maqui berry extract-mixed samples can be maintained up to M3 (50%), which can be used as a pigment in semi-permanent cosmetics.

Colour changes in maqui berry extract-mixed samples

The colour changes in the maqui berry extract-mixed samples were evaluated over time (Figure 10). No significant difference was observed between M1-M4 and the M0 stock solution. The colorimetry results shown in Figure 11 indicate that the L*, a* and b* values of M1-M3 did not significantly vary from M0.

Sample s	M0	M1	M2	M3	M4	M5	M6
Rubber board							
After 3 months							

Figure 10. A comparison of colour changes of maqui berry extract-mixed samples observed over time.

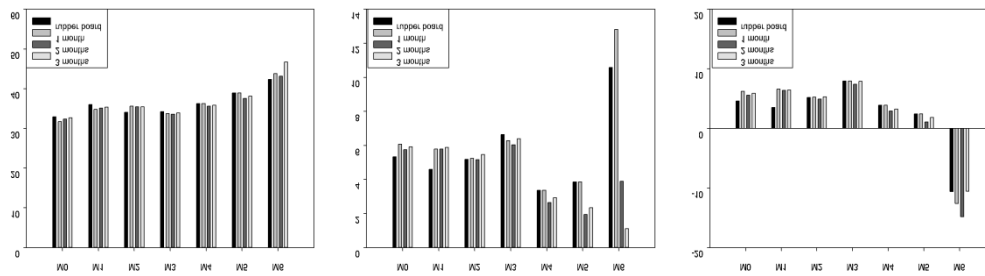


Figure 11. Results of colour difference analysis over time for maqui berry extract-mixed samples.

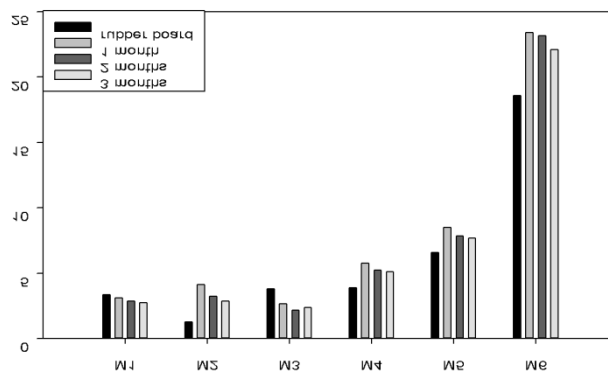


Figure 12. Colour change ΔE^*_{ab} analysis results over time of maqui berry extract-mixed samples.

ΔE^*_{ab} analysis for the maqui berry extract-mixed samples over time (up to three months) showed that the values of M1-M6 were 2.75, 2.88, 2.38, 5.11, 7.66, and 22.1, respectively, indicating 3.00 or below for samples up to M3. Thus, even with time, the stability of maqui berry extract-mixed samples with respect to skin-surface adherence was maintained without a significant difference from the stock solution of the semi-permanent pigment.

Heavy metal and potassium contents in maqui berry extract-mixed samples

The ICP-OES and ICP-MS results of maqui berry extract-mixed samples showed that the levels of heavy metals, Cd, Cr, As, Pb, and V, decreased by 50% or more for samples M1-M3 with 50% maqui berry extract, whereas the K content increased with the increasing amount of maqui berry extract. This is due to the abundance of K in 100% maqui berry extract. The ICP-OES and ICP-MS results for heavy metals and K are illustrated in Figures 13 and 14, respectively.

Dispersion stability of maqui berry extract-mixed samples

In OM of maqui berry extract-mixed samples (Figure 15), a stable dispersion state was observed for samples M1-M4 with up to 70% maqui berry extract, whereas samples M5 and M6 exhibited an unstable state.

Samples	M0	M1	M2	M3	M4	M5	M6
×100							
×400							

Figure 15. OM analysis of maqui berry extract-mixed samples

Cytotoxicity assay of maqui berry extract-mixed samples

The MTT assay of the 50% maqui berry extract-mixed sample (M3) demonstrated cell viabilities of 97.4 ± 1.00 , 98.5 ± 0.60 , 94.6 ± 1.30 , 89.8 ± 1.40 , and $88.7 \pm 1.70\%$ at the test material concentration of 0.01, 0.1, 0.5, 1, and 5 $\mu\text{g/mL}$, respectively (Figure 16). Thus, 80% or higher cell viability was confirmed for up to 5 $\mu\text{g/mL}$ concentration of the M3 sample.

Pigskin pigment-adherence capacity of maqui berry extract-mixed samples

The pigment-adherence capacity of maqui berry extract-mixed samples on the pigskin (Figure 17) indicated that the L*, a* and b* values did not vary significantly for all samples from the stock solution.

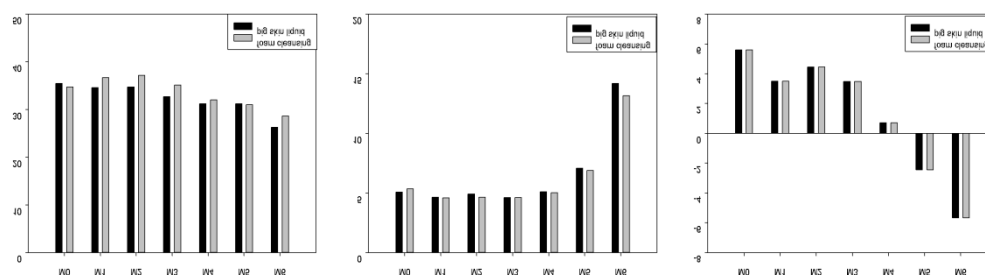


Figure 17. Results of pigment-adherence capacity of maqui berry extract-mixed extract samples on the pick skin.

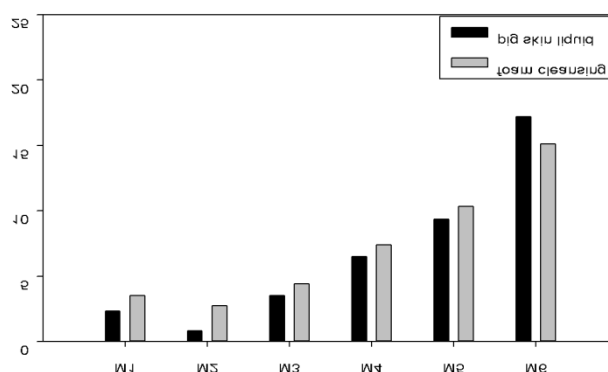


Figure 18. ΔE^*_{ab} analysis for determining the pigment-adherence capacity of the maqui berry extract-mixed samples on the pigskin

In the case of ΔE^*_{ab} analysis of the maqui berry extract-mixed extracts regarding the pigment-adherence capacity on the pigskin (Figure 18), the values of M1-M6 were 3.52, 2.75, 4.40, 7.41, 10.3, and 15.1, respectively, indicating 5.00 or less up to M3. Even after rubbing using constant pressure with a cotton ball soaked in foam cleanser for 10 min, and then with a cotton ball soaked in distilled water, the pigment-adherence capacity on the pigskin remained highly similar to that of the pigment stock solution for maqui berry extract-mixed samples, without significant differences in the L^* , a^* , and b^* values. Thus, the results indicated that the stability of maqui berry extract-mixed samples with respect to the adherence to the surface of the skin can be maintained.

4. CONCLUSION

In this study, an inorganic dye pigment was mixed with varying concentrations of natural extract of maqui berry to verify the potential use of natural pigments in semi-permanent cosmetics that are safe and stable, considering the adverse effects on the skin and the risk of heavy metals associated with semi-permanent cosmetics.

With the confirmed antioxidant and antibacterial capacities, collagenase inhibition activity, and the lack of cytotoxicity, the maqui berry extract was mixed with the stock solution of the semi-permanent cosmetic pigment for subsequent analyses. The colorimetric analysis results showed no significant difference between the stock solution sample and the maqui berry extract-mixed samples 10-50%. Similarly, the colour of the pigskin showed no significant difference in L^* and a^* between the stock solution of the pigment and the 10-50% solutions, although a decreasing trend in b^* was observed. The analysis of colour change in maqui berry extract-mixed samples over time showed that the colorimetric values were similar for the stock solution and samples 10-50% with respect to the L^* , a^* , and b^* values. The ICP-MS analysis revealed that, compared to semi-permanent cosmetic pigments, the level of heavy metals in samples 10-50% was reduced by 50% or more, thereby verifying their potential safety for use as natural pigment products. In addition, the ICP-OES analysis indicated that the content of the mineral K increased as the concentration of the maqui berry extract increased. The OM analysis demonstrated that the dispersion was stable in samples 70%, while the samples of 90% or higher concentration displayed an unstable state. The MTT assay demonstrated 80% or higher cell viability at concentrations up to 5 $\mu\text{g}/\text{mL}$ for 50%. For the pigment-adherence capacity on the pigskin, no significant difference in L^* , a^* , and b^* from the stock solution was found for the extract-mixed samples, suggesting the stable skin adherence of maqui berry extract-mixed samples upon use. Thus, the mixture of a semi-permanent cosmetic pigment and maqui berry extract in this study did not vary notably in colour from the pigment stock solution, while the heavy metal content significantly decreased and the mineral K content increased.

The findings in this study suggest that the semi-permanent cosmetic pigment mixed with maqui berry natural extract can be used as a safer natural product than conventional pigment products. The results are also anticipated to serve as reference data in applied research on cosmetic pigments using various functional natural extracts.

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