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Application of Butterfly Pea Flower Extract in Mask Development

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Abstract: (1) Background: *Clitoria ternatea* (butterfly pea), a plant species belonging to the *Leguminosae* (Fabaceae) family, is useful for medical treatments and has been used in folk medicines and to cure different diseases. The antioxidation ability of the total phenolic compounds of butterfly pea is useful for preserving flavor, and colour and for preventing vitamin destruction in processed foods. In this study, a butterfly pea flower fermentation solution was added to cosmetics as a whiting ingredient. (2) Methods: After the phenolics, flavonoids and ascorbic acid content of the butterfly pea flower extraction had been determined, lactic acid bacteria fermented the extraction. The whitening and moisturizing effect was assayed by SSC3 and NF333 analyzers. (3) Results: This study demonstrated that the butterfly pea flower fermentation solution has free radical scavenging ability, a reducing power in high concentrations, a moisturizing effect, and a whiting effect. (4) Conclusions: The results showed that the butterfly pea flower fermentation solution not only inhibits redness, itching, allergies, and irritation to the skin, but also has antioxidation properties and promotes moisture retention and whitening effects, and the results increase as the concentration increases. Therefore, butterfly bean flowers may be suitable as a raw material for natural beauty care products.

Keywords: butterfly pea flower fermentation solution; moisture retention; whitening

1. Introduction

The butterfly pea flower (*Clitoria ternatea*) originates from subtropical regions and is widely distributed in Africa, Asia, Australia, North America, South America, and the Northwest, Central South, and Southwest Pacific. Butterfly pea, like vine plants, is a perennial climbing plant or herbaceous plant. It is self-pollinated and spreads by seeds. It prefers moist and neutral soil (pH 5.5 to 8.9). Butterfly pea flower is an axillary, solitary, or twin flower. It is harvested in summer. The most apparent feature of the butterfly pea flower is its dark blue petals with a yellow mark. According to Zakaria et al. [1], the flavonoids found in the butterfly pea flowers can reduce infections in the upper respiratory tract. They have been proven to be anti-inflammatory in animal tests and have antioxidative power. Butterfly pea flowers contain about 0.9 mg ash, 8.94 mg soluble minerals, 41.27 mg crude protein, and 29.18 mg soluble carbohydrate per 100 g of dry weight. Terahara et al. [2] used reversed-phase High Pressure Liquid Chromatography (HPLC) to isolate five major structures of anthocyanins, which are delphinidin derivatives that are highly acylated.

Tantituvanont et al. [3] pointed out that the anthocyanins in butterfly pea flowers are mainly delphinidin-glucosides. Anthocyanins are water-soluble macromolecular substances that give orange, red, or blue-violet colour to fruits, vegetables, flowers, leaves, or stems. Saptarini et al. [4] showed that the anthocyanins in butterfly pea flowers appear blue at pH 4, green at pH 9, and yellow at pH 12, making it an acid-base titration indicator. Anthocyanins are susceptible to environmental and chemical influences, including pH variation, ambient temperature, light, oxidation, and enzymes,

making their application in the food industry difficult (Mohamed et al. [5]). Tantituvanont et al. [3] and Mohamed et al. [5] found that the anthocyanins in butterfly pea flower are more stable at low temperatures, and the flowers are purplish red in acidic environments and blue in an alkaline environment. Compared with alkaline environments, the stability and antioxidative activity of the anthocyanins are higher in weakly acidic environments. Rabeta et al. [6] pointed out that the blue flowers of butterfly peas have good free radical scavenging activity and have potential as antioxidants. Rajamanickam et al. [7] suggested that the antioxidative power of the methanol extract of butterfly pea flowers is equivalent to that of L-ascorbic acid.

The butterfly pea flower contains anthocyanins and thus, is a natural antioxidant that can delay the aging of the skin and is good for the skin. Therefore, in this study, butterfly pea flowers extracted by cold water extraction and hot water extraction were analyzed for their total phenolics, flavonoids, and ascorbic acid content as well as their antioxidative power. Fermentation solution from lactic acid bacteria contains lactic acid, peptides, and polysaccharides, which are excellent for skin whitening effects. In this study, butterfly pea flowers were fermented by the lactic acid bacteria in a safe and pollution-free environment. The performance of the fermentation solution was evaluated for its whitening and moisture retention effects in order to determine the value of using a butterfly pea flower fermentation solution in cosmetic applications.

2. Materials and Methods

2.1. Preparation of the Butterfly Pea Flower Fermentation Solution

The Butterfly Pea Flower was kindly provided by bluetopurple Corp. agency (Wujie Township, Yilan County, Taiwan). Take 1 L of the culture medium listed in Table 1 and sterilizer for 1 h at 121 °C at a concentration of 1.2 kg/cm^2. Wait until room temperature is reached and then mix 50.0 g of butterfly bean flowers, 50.0 g of sugar-free soy milk, 5.0 g of lactic acid bacteria (*Lactobacillus acidophilus*, also called strain A, ATCC 4357) and 1 L of deionized water in a UV disinfection chamber before moving it to a shaking incubator for fermentation for 48 h at 30 °C and 125 rpm. After centrifugation at a high speed, take the supernatant and then sterilize it at 121 °C at a concentration of 1.2 kg/cm^2) in the sterilizer for 1 h. Wait until room temperature is reached, and then filter it into the UV disinfection chamber.

Table 1. Butterfly pea flower fermentation solution formula.

Approximate	Per Liter
Proteose Peptone No.3	10.0 g
Yeast Extract	5.0 g
Dextrose	20.0 g
Polysorbate 80	1.0 g
Ammonium Citrate	2.0 g
Sodium Acetate	5.0 g
Magnesium Sulfate	0.1 g
Dipotassium Phosphate	2.0 g

2.2. Determination of the Total Phenolic Content

The total phenolics content measurement was done in accordance with Taga et al. [8]. The method was as follows: Add 0.5 mL of 0.3% HCl to standard caffeic acid (0.001 mg/mL to 1.0 mg/mL) solutions and mix it well. Take 100 µL of the mixture, add 2.0 mL of 2% Na_2CO_3 to the mixture, mix it well, and let it stand for 2 min. Add 0.1 mL of Folin–Ciocalteu agent, and let it stand for 30 min. Repeat the same procedure for each concentration. Measure the OD765 value of each concentration with a spectrophotometer and draw a calibration curve. Replace the caffeic acid with the butterfly pea extract and follow the same procedure to determine the total phenolics of the sample [8].

2.3. Determination of Total Flavonoid Content

The method reported by Jia et al. [9] was revised. Different concentrations of quercetin (6.25–200 mL) were prepared as standard solutions. The OD values of different concentrations were measured to draw a calibration curve and the regression equation was obtained. The method used was as follows: Take 0.5 mL of the sample, add 1.5 mL of pure water, 0.1 mL of 10% aluminum nitrate, 9-hydrate, and 0.1 M of potassium acetate. Then, add 2.8 mL of pure water, mix it well, and let it stand for 40 min. Measure the OD415 value with the spectrophotometer, and then calculate the flavonoid content according to the regression equation of the calibration curve.

2.4. Determination of the Ascorbic Acid Ccontent

Extract 1 mL of the extract with 10 mL of 1% metaphosphoric acid, and then add 9 mL of 2,6-dichloroindo-phenol (DPI) to react. Measure the OD515 value with the spectrophotometer, and draw a calibration curve with different concentrations of ascorbic acid to calculate the ascorbic acid content in the sample [10].

2.5. DPPH Free Radical Scavenging Ability

The method was performed as described by Yamaguchi et al. [11] as follows: Mix 100 µL of the butterfly pea flower extract well with 400 µL of 100 mM Tris-HCl buffer (pH 7.4) and 500 µL of 250 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical ethanol solution in a microcentrifuge tube before placing the tube in a 25 °C thermostatic reactor for 20 min. Transfer the tube to a UV-Vis spectrophotometer to measure the OD517 of the solution. Repeat the procedure three times. Calculate the DPPH free radical scavenging rate with the following formula:

$$\text{DPPH free radical scavenging rate (\%)} = [1 - (\text{OD517 of sample}/\text{OD517 of blank solution}] \times 100\% \quad (1)$$

2.6. Determination of the Reducing Power

Take 2.5 mL of extract, add 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Place the solution in a 50 °C water bath for 20 min. Add 2.5 mL of 10% TCA to the solution after cooling, mix it well, and centrifuge at 3000 rpm for 10 min. Take 5 mL of supernatant, add distilled water and 1 mL of 0.1% ferric chloride, and mix it well. Measure the OD700 value with the spectrophotometer—the higher the OD value is, the better the reducing power is [12].

2.7. Whitening Effect and Moisture Retention Assay

Instrument: three-in-one skin analyzer (SSC3) and NF333 spectrophotometer (Figure 1);

(1) Brand: SSC3, Courage-Khazaka Electronic Gmbh (CK), Köln, Germany;
(2) NF333: NIPPON DENSHOKU, Tokyo, Japan.
(3) Testing methods

- This research protocol was approved by Human Research Ethics Committee of Research Ethics Office of National Taiwan University, protocol number: 201705ES002 (4 October 2018).
- Sampling: The butterfly pea flower fermentation solutions were diluted with distilled water in the following concentrations. Two milliliters of 1.0%, 2.5%, 5.0%, 7.5%, and 10.0% diluted solutions were used.
- Testing spots: forehead and cheek.
- Testing age/skin condition: 18- to 20-year-old females. They are all obtained and indicated this issue for publication.
- Testing environment: thermostatic indoor temperature at 22 °C.
- Testing area: divided into an experimental area and a control area. The right site was the experimental area (for the cleansing mousse with the butterfly pea flower fermentation

solution), and the left side was the control area (for the cleansing mousse without the butterfly pea flower fermentation solution).

- Retention time: the entire face was cleaned and then we waited for 30 min until testing.

Figure 1. Three-in-one skin analyzer and NF333 spectrophotometer.

3. Results

The total phenolics were 185.3 mg/100 g and 239.6 mg/100 g in butterfly pea flower extract for the cold water extraction and hot water extraction, respectively. The flavonoid concentration was 106.9 mg/100 g and 128.3 mg/100 g in butterfly pea flower extract in the cold water extraction and hot water extraction, respectively. The ascorbic acid content was 10.36 mg/100 g in the butterfly pea flower cold water extraction. Because hot water destroys ascorbic acid, the ascorbic acid content was not measured for the hot water extraction, and thus was indicated as "not detected" (N.D., see Table 2). The results showed that hot water can extract more total phenolics and flavonoids. However, ascorbic acid is susceptible to high temperatures, so it cannot be extracted by hot water.

Table 2. Contents of total phenolics, flavonoids, and ascorbic acid in the butterfly pea flower sample.

Sample	Extraction Method	Total Phenolics (mg/100 g)	Flavonoids (mg/100 g)	Ascorbic Acid (mg/100 g)
Butterfly pea flower (*Clitoria ternatea*)	Cold water extraction	185.3 ± 0.096	106.9 ± 0.23	10.36 ± 0.028
	Hot water extraction	239.6 ± 0.081	128.3 ± 0.054	Not Detected

3.1. DPPH Free Radical Scavenging Ability of the Butterfly Pea Flower Extract

DPPH free radicals are stable free radicals containing an odd number of electrons. When they are combined with other free radicals or reduced by antioxidants, DPPH free radicals are scavenged (DPPH +AH→DPPH-H + A), and their color changes from purple to light yellow, which, in turn, reduces their absorbance. The lower the absorbance is, the stronger a sample's DPPH scavenging ability is and the stronger its antioxidation ability is. DPPH ethanol solution has very strong absorbance at 517 nm visible light. Figure 2 shows the DPPH free radical scavenging ability of the butterfly pea flower cold water extraction and hot water extraction. In terms of antioxidative power, the DPPH free radical scavenging ability of the 1 mg/mL butterfly pea flower hot water extraction was about 6.79% and that of the 10 mg/mL extract was about 65.23%. When the concentration of the butterfly pea flower hot water extraction increased to 100 mg/mL, the DPPH free radical scavenging ability increased to 75.69%, which is equivalent to 76% of 1 mg/mL BHT. As for the butterfly pea flower cold water extraction, the DPPH free radical scavenging ability was about 5.39% and 58.23% for 1 mg/mL and 10 mg/mL of extract, respectively. When the concentration of the cold butterfly pea flower extract was increased to 100 mg/mL, the DPPH free radical scavenging ability increased to 63.25%, which is equivalent to 64% of 1 mg/mL BHT. The results showed that the butterfly pea flower hot water extraction had a better DPPH free radical scavenging ability than the cold water extraction. When the concentration of the butterfly pea flower extracts increased from 1 mg/mL to 10 mg/mL, the DPPH free radical

scavenging ability increased. This was true for the butterfly pea flower cold water extraction and hot water extraction. However, when the concentration was higher than 10 mg/mL, the DPPH free radical scavenging ability did not increase linearly, indicating that the DPPH free radical scavenging abilities of the butterfly pea flower extracts were about 60% to 70% of 1 mg/mL BHT (Figure 2).

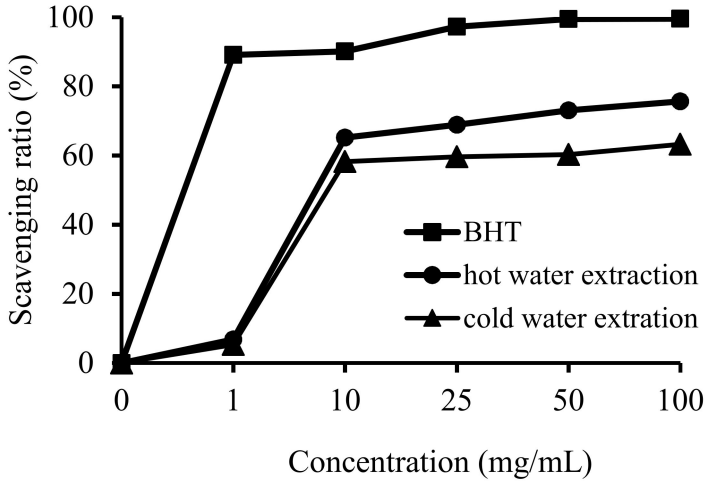

Figure 2. DPPH free radical scavenging ability of the butterfly pea flower extracts.

3.2. Reducing Power of the Butterfly Pea Flower Extracts

The higher the OD_{700} absorbance is, the better the reducing power is. In this study, 1 mg/mL, 10 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL butterfly pea flower cold water and hot water extractions were used to determine the reducing power of butterfly pea flower extract, and this was compared with the standard BHT. The results showed that the OD700 values of the butterfly pea flower hot water extraction were about 0.5–2.3, and those of the butterfly pea flower cold water extraction were about 0.4–2.0, indicating that the butterfly pea flower hot water extraction had a higher reducing power. At the concentration of 100 mg/mL, the reducing power of the butterfly pea flower hot water extraction was about 96% of the standard BHT. However, that of the butterfly pea flower cold water extraction was only about 83% of the standard BHT (Figure 3).

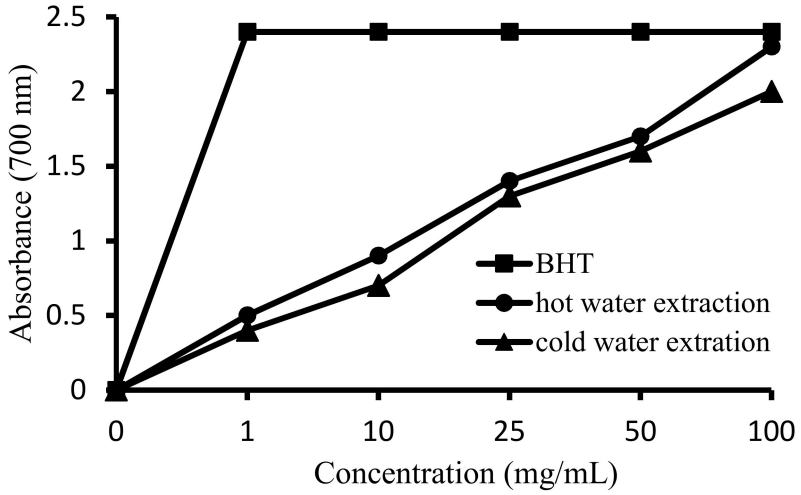

Figure 3. Reducing power of the butterfly pea flower extract.

3.3. Moisturizing Effect of the Butterfly Pea Flower Fermentation Solution

The butterfly pea flowers were fermented by lactic acid bacteria. The fermentation solution was diluted with distilled water to 1.0%, 2.5%, 5.0%, 7.5% and 10.0% diluents. The diluents were

the experimental group, whereas distilled water was the control group. After the assessment by the moisture instrument, the moisture improvement rates after 14 days were 7.87% for the 1.0% diluent, 11.20% for the 2.5% diluent, 15.15% for the 5.0% diluent, 17.91% for the 7.5% diluent, and 18.11% for the 10.0% diluent. The moisture improvement rates after 28 days were 10.20% for the 1.0% diluent, 14.28% for the 2.5% diluent, 15.91% for the 5.0% diluent, 19.63% for the 7.5% diluent, and 20.32% for the10.0% diluent (see Table 3 and Figure 4).

Table 3. Moisture values of the fermentation solutions at different concentrations after 14 days and 28 days of use.

Concentration of Butterfly Pea Flower Fermentation Solution	Day 14		Day 28	
	Control (%)	Experimental (%)	Control (%)	Experimental (%)
1.0%	12.7	13.7	12.2	13.5
2.5%	12.5	13.9	12.25	14
5.0%	13.2	15.2	12.25	14.2
7.5%	13.4	15.8	12.12	14.5
10.0%	13.8	16.3	12.3	14.8

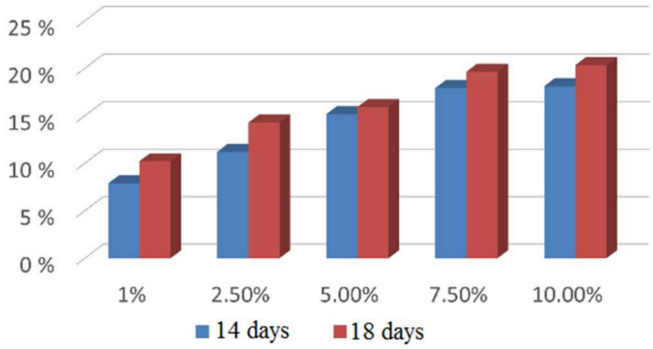

Figure 4. Moisture improvement rates (%) of the fermentation solutions at different concentrations after 14 days and 28 days of use.

3.4. Whitening Effect of the Butterfly Pea Flower Fermentation Solution

The whitening improvement rate after 28 days was 7.99% for the 1.0% diluent; 10.53% for the 2.5% diluent; 18.50% for the 5.0% diluent; 22.21% for the 7.5% diluent; and 29.97% for the 10.0% diluent (see Table 4 and Figure 5).

Table 4. Whitening values of the fermentation solution at different concentrations after 28 days of use.

Concentration of Butterfly Pea Flower Fermentation Solution	Day 28	
	Control (%)	Experimental (%)
1.0%	64.63	69.79
2.5%	63.87	70.60
5.0%	62.84	74.47
7.5%	65.02	79.46
10.0%	64.21	83.45

3.5. Formula Design and Product Development

With the rise in environmental awareness, many people have started to pay attention to the safety of skincare products. We have all heard about the serious pollution and damage imposed upon the environment by skincare products made of chemical raw materials, such as environmental hormones. Such skincare products also harm the human body to various degrees. Therefore, natural and environmentally-friendly products have become preferred by consumers.

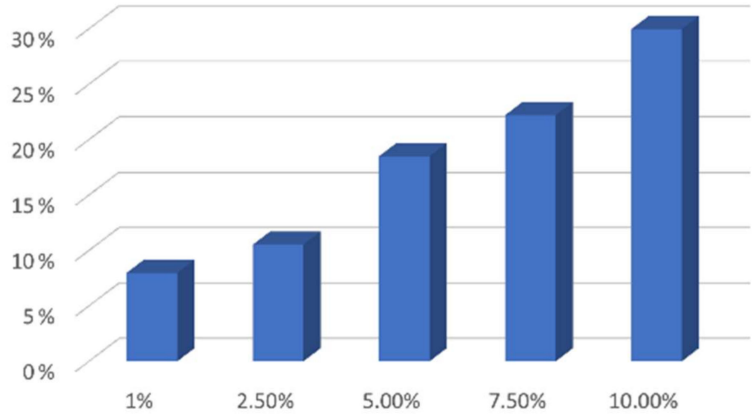

Figure 5. Whitening improvement rates of the fermentation solutions at different concentrations after 28 days of use.

The above experiments confirmed that the butterfly pea flower fermentation solution has whitening, moisture retention, and anti-aging effects. It is suitable for use as a raw material in skin care products. Therefore, we have preliminarily designed a mask formula containing sodium bicarbonate, hot spring water, and butterfly pea fermentation solution, and have looked for a manufacturer to develop the product through industry–academia collaboration.

Good essence is the soul of a skin care product. Exquisite ingredients produce remarkable effects. From design to material selection, we failed more than 10 times before coming up with the mask formula presented in Table 5. After 30 days of use, the hot spring mask containing 6% butterfly pea flower fermentation solution can improve moisture retention by 20.35% (Table 6) and enhance the whitening effect by 18.34% (Table 7). With respect to skin irritation, there was no irritation after 24 h of patch testing. In an accelerated aging test, there was no deterioration after the sample was tested in a thermostat chamber at 40 ± 2 °C with a relative humidity of 75 ± 5%.

Table 5. Butterfly pea flower brightening mask containing hyaluronic acid and hot spring water.

No.	Chinese Name	%
1	Pure water	To 100.00
2	Hot spring water	6.00
3	Fermented butterfly pea flowers	6.00
4	Glycerin	2.00
5	Propylene glycol	0.80
6	Cucumis sativus (Cucumber) fruit extract	0.60
7	Saccharomyces lysate extract	0.45
8	Acrylates/ C10-30 alkyl acrylate crosspolymer	0.12
9	Phenoxyethanol	0.17
10	Xanthan gum	0.06
11	Chlorphenesin	0.20
12	Arginine	0.03
13	Sodium hyaluronate	0.30
14	Hydrolyzed hyaluronic acid	0.15
15	Allantoin	0.10
16	Ammonium acryloyldimethyltaurate/VP copolymer	0.10
17	Aloe barbadensis leaf juice	0.10
18	Panthenol	0.05
19	Glycyrrhiza glabra (Licorice) root extract	0.05
20	Fragrance	0.01
21	Capryl glycol	0.10

Table 6. Average moisture retention effect of 20 subjects using the butterfly pea flower hot spring water mask.

Moisture Retention	Day 30	
	Control (%)	Experimental (%)
	51.6	62.1

Table 7. Average whitening effect of 20 subjects using the butterfly pea flower hot spring water mask.

Whitening Effect	Day 30	
	Control (%)	Experimental (%)
	58.38	69.09

4. Discussion

The experimental data show that the organic butterfly pea flower extract or fermentation solution not only did not cause redness, itching, allergy, or irritation to the skin but also improved moisture retention and had whitening effects, and these effects increased as its concentration increased. As a result, the butterfly pea flower fermentation solution can be added to cosmetic formulas as a natural raw material of skin care products. The outcome of the industry–academia collaboration can be added to teaching materials of various courses, such as cosmetics preparation, projects, and on-campus internships, and can enhance the students' interests and skills in cosmetics preparation and achieve the effects of diverse learning. The teachers and students will also fulfill their local social responsibility, assist in the innovation and development of the local industry, and be pioneers of the University Social Responsibility (USR) Project. The university can also reuse the resources and make green living a reality in order to develop a green economy, implement ecological concepts, and make life full of joy and blessing.

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