Phytochemical Screening And Sun Protection Factor (Spf) Evaluation Of Butterfly Pea Flower (*Clitoria Ternatea* L.) Extract Lip Tint Product

Septiana Laksmi Ramayani[1)*], Yenni Arumsari[2)], Definingsih Yuliastuti[1)]

[1] Lecturer of Pharmacy Department, Poltekkes Kemenkes Surakarta, Klaten, Indonesia

[2] Student of Pharmacy Diploma Program, Pharmacy Department, Poltekkes Kemenkes Surakarta, Klaten, Indonesia

*email: septianaLR@gmail.com

Abstract

Lip tint is a cosmetic product that adds color to the lips while protecting them from the harmful effects of UV rays and free radicals. One of the natural colorants with sunscreen activity that can be used in lip tint products is the butterfly pea (Clitoria ternatea L.) flower. The goal of this study was to investigate the phytochemical compounds and SPF activity of butterfly pea flower extract lip tint products at varying extract concentrations. The study was conducted using an experimental design with a Complete Randomized Design (CRD). Butterfly pea flowers were extracted using 96% ethanol and HCl through maceration. The extract was then formulated into lip tint products at concentrations of 5%, 10%, and 20%. Phytochemical screening was performed on the butterfly pea flower extract lip tint products, revealing the presence of alkaloids, flavonoids, saponins, terpenoids, and phenol-tannins. The SPF values of the lip tint products were determined in vitro using the Mansur method. The in vitro SPF test results showed that at a concentration of 5%, the SPF value of the lip tint product was 1.618162, which is considered low. At a concentration of 10%, the SPF value increased to 4.770857, which is classified as medium. At a concentration of 15%, the SPF value rose to 6.1224069, which is classified as high. Statistical analysis indicated significant differences among the three concentrations ($p<0.05$), indicating that the concentration of butterfly pea flower extract affects the SPF value of lip tint products.

Keywords: butterfly pea flower, c*litoria ternatea* L., lip tint, SPF

1. INTRODUCTION

Indonesia, situated on the equator, receives ample sunlight throughout the year. However, exposure to UVA and UVB rays can have detrimental effects on the skin, including the lips, which may become parched, chapped, and lose color owing to the cracking of the keratinized surface layer (Ardiansyah *et al*., 2022).

To prevent the adverse consequences of UV rays, experts recommend using lip tint products that incorporate Sun Protection Factor (SPF) and antioxidants. Such products can help guard against the damage caused by UV rays. It is important to note, however, that some cosmetic lip tint products may contain synthetic antioxidant compounds like Butyl Hydroxy Toluene (BHT) and synthetic colorants, which may lead to carcinogenesis (Nurmalasari *et al*., 2016).

Therefore, it is strongly advised to avoid products that contain such compounds and to opt for those that contain natural ingredients, including botanical extracts, that are known to be safe and effective in promoting healthy and radiant lips.

Researchers have developed a lip tint using the active substance component of butterfly pea (*Clitoria ternatea* L.) flowers. The butterfly pea flowers contain natural antioxidant compounds, including a flavonoid chemical content of 0.493% and an anthocyanin content of 0.1927% (Hawari

et al., 2022). These compounds have the potential to act as photoprotective agents, absorbing harmful UV rays.

The researchers found that the butterfly pea flower extract at a concentration of 300 ppm has an SPF activity of 8.42, which falls within the maximum category (Paongan & Vifta, 2022). This has led them to evaluate the Sun Protection Factor (SPF) value of the butterfly pea (*Clitoria ternatea* L.) flower extract lip tint in vitro using the UV-Vis spectrophotometric method.

2. METHOD

Type of research

This experimental research provides treatment by deliberately intervening in samples to determine the effect or relationship between two or more variables (Rukminingsih *et al*., 2020).

Complete Randomized Design (CRD) was used in this study with varying concentrations of butterfly pea extract (5%, 10%, and 15%) and three replications. The method used in this research is quantitative as the researchers present the findings in the form of numbers.

Materials and Tools

The apparatus used in this research were analytical balance (Labex)®, stirring rod, glass jar, cup, water bath (Equitron)®, aluminum foil, test tube, filter paper, mortar and stamper, lip tint container, spoon, drop pipette, cuvette, drop pipette, micropipette, UV-Vis spectrophotometer, volumetric flask (Pyrex)®, and measuring cup (Pyrex)®.

Dried butterfly pea (*Clitoria ternatea* L.) flower, 96% ethanol, 5% potassium hydroxide (KOH) solution, butterfly pea flower extract, methylparaben, propylene glycol, isopropyl myristate, glycerin, sorbitol, PEG-40 hydrogenated, and rose essential oil, hydrochloric acid (HCl), sulphuric acid (H_2SO_4), Mayer reagent, iron (III) chloride ($FeCl_3$) were the materials used in this research.

Methods

a. Sample Determinations

Dried butterfly pea flowers were obtained from Babadan, Purwomartani, Kalasan, Sleman Regency, Yogyakarta, Indonesia. Samples were determined at the Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT) Tawangmangu, Karanganyar Regency, Central Java.

b. Grinding

Butterfly pea flowers were sorted using a dry method, then grinded to make butterfly pea flower powder. The resulting powder was sieved through a 60/80 mesh (Ardiansyah *et al*., 2022).

c. Extracting Process

500 grams of butterfly pea flower powder was macerated for 3 days with 3750 mL of 96% ethanol and hydrochloric acid (HCl). After filtration, the remaining pulp was then remacerated for 2 days with the same solvent 1250 mL. The obtained solution was then evaporated using a water bath with a temperature of 45°C.

Table 1. Formula of Butterfly Pea Flower Extract Lip Tint

Materials	Formulas (%)		
	F1	F2	F3
Butterfly pea flower extract	5	10	15
Methylparaben	0.3	0.3	0.3
Propylene glycol	15	13	11
Isopropyl myristate	20	19.5	18.5
Glycerin	19.7	18	18.2
Sorbitol	20	19.5	18.5
PEG-40 hydrogenated	20	19.5	18.5
Rose essential oil	qs	qs	qs

d. Butterfly Pea Flower Extract Lip Tint Formulation

Methylparaben was dissolved in propylene glycol then added with isopropyl myristate, followed by glycerin and sorbitol, and stirred until a homogenous mixture was obtained. Next, PEG-40 hydrogenated was added to the mixture, along with butterfly pea flower extract, and stirred until a homogenous mixture was obtained.

e. Preliminary Test of Chromophore Groups

2 grams of lip tint product was dissolved in 10 mL of distilled water and heated for 30 minutes. After heating, the mixture was filtered, and 3 drops of KOH were added. A chromophore group positive produced an intensive yellow to brownish-red color.

f. Phytochemical Screening Tests

1. Alkaloid

40 mg of the lip tint product was dissolved in a few drops of 1% HCl, followed by the addition of 1 mL of Mayer reagent. A positive reaction is indicated by the presence of a precipitate or cloudy solution (Hanani, 2016).

2. Flavonoid

40 mg of the lip tint product was added to 100 mL of hot distilled water, boiled for 5 minutes, and filtered. 5 mL of filtrate mixed with 0.05 mg of magnesium (Mg) powder and 1 mL of concentrated HCl. The solution was then shaken vigorously. A positive result is indicated by a color change to red, yellow, or orange (Hanani, 2016).

3. Saponin

40 mg of the lip tint product was added to 10 mL of distilled water, shaken for 1 minute, and mixed with 2 drops of HCl 1N. A positive result is indicated by the stable foam remaining for ± 7 minutes (Hanani, 2016).

4. Terpenoid

100 mg of the lip tint product dissolved using 10 mL of distilled water. Then, 2 mL of dissolved lip tint was mixed with 3 drops of concentrated HCl and 1 drop of concentrated sulphuric acid (H_2SO_4). A positive result is indicated by the formation of red or purple color (Hanani, 2016).

5. Phenol-Tannin

A total of 40 mg of the lip tint product was dissolved in 4 mL distilled water, Then, 2 mL of dissolved lip tint was mixed with 1 mL of iron (III) chloride ($FeCl_3$) 10%. A positive reaction is indicated by the formation of a dark blue or greenish-black color (Hanani, 2016).

6. Anthraquinone

A total of 50 mg of of the lip tint product was dissolved in 10 mL of distilled water heated for 5 minutes and filtered. 3 mL of solution was put into 2 test tubes. The second tube was mixed with NaOH 1N. A positive result is indicated by the formation of a red solution (Hanani, 2016).

g. Determining Lip Tint SPF Value

A 2500 ppm solution was made by dissolving 25 mg of each lip tint product with concentrations of 5%, 10%, and 15% in 96% ethanol p.a. in a 10 mL volumetric flask. UV-Vis spectrophotometry was used to measure the absorbance at a wavelength of 290-320 nm, and the absorbance values were then used to calculate the SPF value using the Mansur method, as shown in equation (1) and Table 2 (Ardiansyah *et al*., 2022).

$$\text{Spectrophotometer SPF} = \text{CF} \times \sum_{290}^{320} EE \times \text{I} \times \text{Abs} \quad (1)$$

EE = Erythema Effect Spectrum
I = Light Intensity Spectrum
Abs = Sample absorbance
CF = Correction Factor (10)

The EE x I value to determine the SPF value using the Mansur method can be seen in Table 2.

Table 2. EE x I Values with Wavelengths of 290-320 nm

Wavelength (nm)	EE x I
290	0,0150
295	0,081
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1

3. RESULT AND DISCUSSION

Butterfly pea (*Clitoria ternatea* L.) flowers were obtained from Babadan, Purwomartani, Kalasan, Sleman Regency, Yogyakarta. Babadan is located at an altitude of approximately 127 meters above sea level, which is classified as lowland. Butterfly pea flowers can bloom in lowland areas with an altitude of 1-1800 MASL and an annual rainfall of 2000 mm/year, making this area suitable for butterfly peas to grow (Hawari *et al*., 2022).

Butterfly pea flowers were dry sorted to ensure they were free from unwanted materials and met the standards (Damayanti *et al*., 2020). Then, butterfly pea flowers were grinded and sieved to reduce their particle size and ensure uniform particles of powder.

The standard sifting process involves the use of mesh No. 60/80, which is effective within the 60-100 range. However, when the powder is too fine, the extraction process becomes complex. This is because fine powders reduce the space between cells, making it difficult for the invigorating liquid to penetrate the powder. Thus, the recommended mesh sieve for the sieving process is No. 60. (Rekayasa *et al*., 2020).

Organoleptic tests were performed on the powder, resulting in a purple powder with a distinct aroma of butterfly pea flower. The natural purple color of the butterfly pea flower gives the powder its purple color. This result aligns with existing literature on purple powder and the distinctive aroma of butterfly pea flowers (Paongan & Vifta, 2022). Table 3 displays the organoleptic properties of the butterfly pea flower powder.

Table 3. Organoleptic Properties of Butterfly Pea Flower Powder

Parameter	Result
Color	Purple
Odor	Distinctive butterfly pea aroma
Physical properties	Powder

A thermogravimetric method using a crus tool was employed to measure the water content in butterfly pea flower powder. The test result indicated 8.78% water content in the powder, which is below the 10% limit stated by the Indonesian Ministry of Health, and considered good. Table 4 presents the outcomes of the water content test for butterfly pea flower powder.

Table 4. Water Content Test Result

Parameter	Result	Standard
Water Content Test	8,78%	<10%

The maceration method was selected as an extraction technique due to its simplicity and ability to avoid damage to thermolabile compounds. The solvent used in this process is a mixture of 96% ethanol and 1% hydrochloric acid (HCl). Ethanol is chosen for its non-polar properties (ethyl groups) and polar properties (hydroxy groups). To create an acidic environment in the maceration method, 1% HCl was added, because anthocyanin compounds are known to be more stable in acidic solutions (Damayanti *et al*., 2020). The yield obtained was 19.85%, and the resulting color was dark purple. Table 5 presents the results of the butterfly pea flower extract.

Table 5. Butterfly Pea Flower Extract Properties

Parameter	Result
Powder Weight	500 grams
Extract Weight	99,25 grams
Yield	19,85%
Color	Dark Purple
Odor	Distinctive butterfly pea aroma

A chromophore group test was conducted to determine the presence of UV light in a sample and detects the presence of auxochrome groups with free pairs. The results of the test showed an intense color change from purple to reddish yellow, indicating the presence of chromophore groups in the sample. The color change was observed in Figure 1.

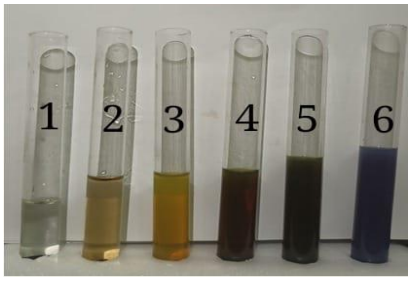

Figure 1. Qualitative Test of Chromophore Groups

Desc :1. Quercetin,2. Quercetin + KOH, 3. FI + KOH, 4. FII + KOH, 5.FIII + KOH, 6. Lip Tint

The lip tint made from the butterfly pea flower extract is a semi-solid product that has a blackish-purple color and a scent of butterfly pea. A phytochemical screening test was performed on the lip tint to determine the content of secondary metabolite compounds. According to the results of the test, the butterfly pea flower extract lip tint contains alkaloids, flavonoids, saponins, terpenoids, and phenol-tannin compounds, as shown in Table 6.

Table 6. Phytochemical Screening Test of Butterfly Pea Flower Extract Lip Tint Product

Secondary metabolites	Reagent	Result	
Alkaloid	Mayer	White sediment	+
Flavonoid	Mg Powder + HCl	Pink solution	+
Saponin	Aquadest	Stable foam	+
Terpenoid	HCl conc +H_2SO_4 conc	Pink solution	+
Phenol-Tannin	$FeCl_3$	Brown black solution	+
Anthraquinone	NaOH 1N	Yellow solution	-

The sample's absorbance was determined using a wavelength of 290-320 nm. UVB wavelengths fall in the erythmogenic region, which may cause sunburn. Figure 2 displays a graph that illustrates the relationship between the wavelength and absorbance value of the butterfly pea flower extract lip tint product. The highest absorbance value is at 290 nm while the lowest is at 320 nm. This indicates as the wavelength increases, the absorbance decreases. This happens because the energy is inversely proportional to the wavelength (Paongan & Vifta, 2022).

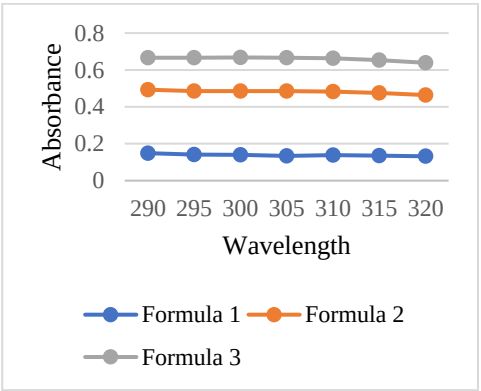

Figure 2. Wavelength and Absorbance Value Graph

The SPF values were obtained at different concentrations 5% (F1), 10% (F2),

and 15% (F3). The SPF values for each concentration are 1.618162 (F1), 4.770857 (F2), and 6.1224069 (F3). Formula 1 has a low sunscreen ability (2-4). Formula 2 has moderate sunscreen ability (4-6), and Formula 3 has extra sunscreen ability (6-8). The highest SPF value was found in Formula 3, which had a concentration of 15% butterfly pea flower extract. The results of the SPF value of butterfly pea flower extract lip tint products can be seen in Table 5.

Table 5. SPF Value of Butterfly Pea Flower Extract Lip Tint

Formula	SPF Value			
	Rep 1	Rep II	Rep III	Average ± SD
1	1.374	1.595	1.884	1.618 ±0.256[a]
2	4.838	4.299	5.174	4.770 ±0.441[b]
3	6.644	5.833	5.888	6.122 ±0.452[c]

Different superscript indicates $p<0.05$

The Post Hoc Test results indicate a significant difference among treatment outcomes for each formula, F1 (5%), F2 (10%), and F3 (15%). The p-value was less than 0.05, leading to the rejection of H0. The analysis of the data showed significant differences, which supports the accepted hypothesis H1. This hypothesis suggests that the various concentrations (5%, 10%, and 15%) of butterfly pea (*Clitoria ternatea* L.) flower extract affect the Sun Protection Factor (SPF) activity value of lip tint products.

Table 5 indicates that Formula 3 has the highest SPF activity in the extra category, while Formula 1 is in the low category and Formula 2 is in the medium category. The concentration of butterfly pea flower extract used in the lip tint product affects its SPF value. This means that the higher the concentration of butterfly pea flower extract in the lip tint product, the higher the SPF value will be. A study by Octasari *et al*. (2021) found that the higher the amount of metabolite compounds, the higher the SPF activity. This is consistent with previous research that showed that a higher concentration of blue porterweed (*Stachytarpheta jamaicensis* (L.) Vahl.) leaves in blue porterweed leaf extract hydrogel products leads to faster diabetic wound healing ability. (Octasari *et al*., 2021).

4. CONCLUSION

The phytochemical screening test results revealed that the butterfly pea flower extract lip tint product contains alkaloids, flavonoids, saponins, terpenoids, and phenol tannins. The SPF values obtained at concentrations of 5% (F1), 10% (F2), and 15% (F3) were respectively 1.618162 SPF, 4.770857 SPF, and 6.1224069 SPF. These values indicate Formula 1 has a low sunscreen ability, Formula 2 has a moderate sunscreen ability, and Formula 3 has an extra sunscreen ability (6-8). The significance level (p-value) of the three concentrations was less than 0.05, indicating a significant difference.

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