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Analysis of Rhodamine B Content in Eyeshadow Cosmetic Preparations with UV-Vis Spectrophotometer and Butterfly Pea Flower (*Clitoria ternatea L.*) Extract

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Abstrak

Di era modern, menjaga penampilan agar terlihat menarik adalah hal yang penting. Banyak orang di luar sana berlomba-lomba untuk mendapatkan penampilan yang paling menarik. Banyak pula yang berlomba-lomba mengeluarkan biaya untuk memperbaiki penampilannya, seperti dengan menjalani berbagai perawatan dan membeli berbagai macam sediaan kosmetik. Salah satu jenis kosmetik yang paling sering digunakan oleh orang-orang adalah *eyeshadow*. Sebagai kosmetik dekoratif, tentu saja *eyeshadow* memiliki kandungan pewarna. Namun, penyalahgunaan pewarna berbahaya dalam sediaan eye shadow masih saja ditemukan, seperti penggunaan pewarna Rhodamin B, hal ini dikarenakan sebagian besar palet *eyeshadow* memiliki warna dasar merah. Oleh karena itu, penulis menyadari pentingnya penelitian untuk membedakan kosmetik eyeshadow yang mengandung Rhodamin B dengan yang tidak, dengan menggunakan indikator bahan alami, mengingat keanekaragaman hayati Indonesia yang melimpah. Rhodamin B dapat dideteksi menggunakan antosianin, yang merupakan pigmen alami dalam berbagai tanaman, salah satunya adalah bunga telang. Hasil pengamatan visual menggunakan ekstrak bunga telang kemudian akan dikonfirmasi menggunakan Spektrofotometer UV-Vis. Hasil analisis kualitatif menunjukkan bahwa semua sampel *eyeshadow* positif mengandung Rhodamin B, yang ditunjukkan dengan perubahan warna ekstrak bunga telang dari biru menjadi ungu dan kemudian menjadi merah muda. Hasil pemeriksaan kuantitatif menunjukkan bahwa konsentrasi Rhodamin B dalam lima sampel berkisar antara 0,419 mg/L hingga 15,891 mg/L. Menurut BPOM, produk kosmetik tidak boleh mengandung Rhodamin B (⩽ 0 mg/L).

Kata kunci : Bunga Telang, *Eyeshadow*, Pewarna, Rhodamin B, Spektrofotometer UV-Vis

Abstract

In the modern era, maintaining a good appearance is crucial. Many people are out there vying for the most outstanding looks. Many are also eager to spend money to improve their appearance, such as by undergoing various treatments and purchasing numerous cosmetic preparations. One type of cosmetics most often used by people is eyeshadow. As a decorative cosmetic, of course, eyeshadow has colouring content. However, Rhodamine B is still misused, a dangerous dye in eye shadow preparations, because most eye shadow palettes have a red base colour. As a result, the author recognizes the importance of researching to differentiate eyeshadow cosmetics containing Rhodamine B from those that do not, using natural ingredient indicators, given Indonesia's abundance of biodiversity. Rhodamine B may be detected using anthocyanins, natural pigments in various plants; one is butterfly pea flowers. The results of visual observations of the reaction of butterfly pea flower extract and cosmetic samples will then be confirmed using a UV-Vis Spectrophotometer. The qualitative analysis revealed that all eyeshadow samples tested positive for Rhodamine B, as shown by the colour change in the butterfly pea flower extract from blue to purple to pink. The quantitative examination revealed that Rhodamine B concentrations in five samples ranged from 0.419 mg/L to 15.891 mg/L. According to BPOM, cosmetic products should not include Rhodamine B (⩽ 0 mg/L).

Keyword : Butterfly Pea Flower, Colorant, Eyeshadow, Rhodamine B, UV-Vis Spectrophotometer

I. INTRODUCTION

In the modern era, maintaining a good appearance is crucial. This is the root source of beauty privilege in society, where societal standards often prioritize people perceived as attractive (Aprilianty, Komariah and Abdullah, 2023). Many people are out there vying for the most outstanding looks. Many are also eager to spend money to improve their appearance, such as by undergoing various treatments and purchasing numerous cosmetic preparations.

The Food and Drug Supervisory Agency of the Republic of Indonesia defines cosmetics as materials or objects applied to the exterior layers of the body, including the skin, external genitalia, nails, hair, lips, teeth, and mucous membranes of the mouth. Their primary function is to generate fragrance, clean, adjust the appearance, improve body odour, and keep the body in excellent condition. Cosmetics are classified into two groups based on their intended use: decorative cosmetics (make-up) and dermatological cosmetics (skincare). Decorative cosmetics are intended to improve aesthetics and conceal skin defects, resulting in a more attractive look and favourable psychological effects, such as enhanced self-confidence. At the same time, skin care cosmetics are intended to keep skin healthy and clean (Wasitaatmadja, 1997; Wulan, 2017).

Eyeshadow is one kind of decorative makeup widely used by people in general. According to Agustina and Wahini (2015), eyeshadow, a decorative cosmetic that provides colour, shadow, and glittering effects to the eyes, must have colouring agents in the product composition (Dwiwulandari, Darsono and Wijaya, 2018). Meanwhile, Tranggono *and* Latifah (2007) stated that eyeshadow, which is one of several cosmetic variants used to enhance the appearance of the eyelids or the area under the brows, necessitates the use of safe materials in production and careful application because it is applied to sensitive skin, particularly on the upper eyelid.

Cosmetics comprise efficacious active ingredients and other ingredients such as coloring agents and fragrances. When these ingredients are mixed, they must follow the rules for making cosmetics, which have been reviewed from various aspects of cosmetic manufacturing technology such as pharmacology, pharmacy, chemical engineering, and others (Nabilah, Herawati and Siti, 2020). Cosmetic formulations generally incorporate two primary types of dyes: those that are insoluble in liquids and those that are soluble in liquids (Anastasia and Desnita, 2023).

It is commonly contended that the colorants employed in decorative cosmetic formulations can be further classified into two distinct categories based on their origin and chemical composition: natural dyes and synthetic dyes. Natural dyes are derived from plant, animal, or mineral sources and are often considered more environmentally friendly and potentially safer for human use. In contrast, synthetic dyes are chemically engineered compounds that offer a broader range of colors and greater stability but may raise concerns regarding toxicity and environmental impact (Tranggono and Latifah, 2007).

Citing the Decree of the Director General of Drug and Food Control Number 00386/C/SK/II/90,

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it is prohibited to use certain dyes, such as orange K1, red K3, red K4, red K10 (Rhodamine B), and red K11, in pharmaceuticals, food, or cosmetic products due to their harmful nature. According to Minister of Health Regulation No. 239/Menkes/Per/V/85, Rhodamine B is a synthetic dye frequently employed in the textile and paper sectors. Hence, its use in cosmetics and food preparations is strictly forbidden, even in tiny concentrations.

Red dyes K10 and K3 are carcinogenic and are frequently used in decorative cosmetics, including eyeshadow (Dian, 2022). Misuse of Rhodamin B in eyeshadow preparations occurs since most eyeshadow palettes have red as their base hue (Suyudi *et al.*, 2022). Unfortunately, numerous cosmetics containing Rhodamine B continue circulating freely on the market. According to the Food and Drug Supervisory Agency (BPOM) in Press Release Number HM.01.1.2.12.23.50 dated December 8, 2023, concerning the Findings of Traditional Medicines and Health Supplements Containing BKO (chemical drugs) and Cosmetics Containing Prohibited/Hazardous Materials in 2023, hazardous or prohibited substances that are often found in cosmetics include retinoic acid, mercury, and hydroquinone in facial cream products, as well as red dyes K10 (Rhodamine B) and red K3 in decorative cosmetics.

As a result, the author recognizes the importance of researching to differentiate eyeshadow cosmetics containing Rhodamine B from those that do not, using natural ingredient indicators, given Indonesia's abundance of biodiversity. Rhodamine B may be detected using anthocyanins, which are natural pigments in various plants. Butterfly pea flowers (Clitoria ternatea L.), known to contain anthocyanins with blue pigments (Rifqi, 2021), will be used as a natural indicator to detect Rhodamine B levels in eyeshadow. The results of visual observations of the reaction of butterfly pea flower extract and cosmetic samples will then be confirmed using a UV-Vis Spectrophotometer.

II. METHODS

This research is experimental because it is being done in a laboratory. The research approaches employed include qualitative and quantitative analysis.

Tools and Materials

The materials required for this research include UV-Vis Spectrophotometer Single Beam (Shimadzu UV-1280), analytical balance (Shimadzu ATX224), micropipette (dragon lab), beaker glass (herma), measuring flask (herma), measuring cup (herma), Whatman No.1 filter paper, 60 mesh sieve, vial bottle, and dropper pipette.

The materials required for this research include ethanol pro-analysis (thermo fisher), methanol pro-analysis (merck), HCl 4M (SAP Chemicals), HCl 2M (SAP Chemicals), NaOH 2M (SAP Chemicals), fresh butterfly pea flowers, Rhodamin B (as a control), distilled water (bratachem), and eyeshadow samples from various brands.

E-ISSN : 2715-0518
P-ISSN : 2715-3053

Samples Preparation

Approximately 2 grams of the eyeshadow sample was transferred to a beaker, to which seven drops of 4M hydrochloric acid (HCl) and 12 mL of methanol pro-analysis were added. The mixture was then homogenized. The resulting solution was filtered, and the process was repeated until the filtrate was clear. The clear filtrate was collected in a 20 mL volumetric flask, and methanol was added to reach the calibration mark, followed by thorough homogenization. Subsequently, 0.8 mL of this filtrate was pipetted into a 10 mL volumetric flask, methanol pro-analysis was added to the mark, and the solution was homogenized to prepare the dye solution, which was then used for qualitative and quantitative analyses (Taupik, Mustapa and Gonibala, 2021).

Qualitative Analysis

1) Plants Species Determination

The plants used for previous research were collected first for determination testing. Determination was carried out to find out the truth of the plants to be studied, avoid errors in collecting materials, and avoid the possibility of mixing the plants to be studied with other plants (Klau and Hesturini, 2021). Determination of the butterfly pea flower (Clitoria ternatea L.) was carried out at the Center for Information and Development of Traditional Medicine, University of Surabaya.

2) Extraction Methods of Butterfly Pea Flower

Using a measuring cup, measure 80 millilitres of ethanol pro-analysis and weigh 20 grams of fresh butterfly pea blossoms. Then, it is extracted by macerating it for one 24-hour period at room temperature and in a dark area (Yuliantini and Rahmawati, 2019).

3) Selectivity

The selectivity test in this study involved visually observing colour changes in the butterfly pea flower extract following the addition of a positive control (Rhodamine B standard) and a negative control (methanol). The observations were validated using a UV-Vis spectrophotometer, measuring at a wavelength of 545 nm (Yuliantini and Rahmawati, 2019).

4) Limit of Detection

The limit of detection was established by preparing a series of Rhodamine B dilutions at concentrations of 1, 5, 10, 25, 50, 100, 250, and 500 mg/L. Subsequently, these dilutions were analyzed using butterfly pea flower extract and evaluated visually (Yuliantini and Rahmawati, 2019).

5) Precision

Precision was assessed by repeatedly measuring the absorbance of Rhodamine B at its maximum wavelength. This procedure was conducted six times to ensure the reproducibility and reliability of the data. Following these measurements, the Relative Standard Deviation (%RSD) value was calculated to quantify the precision of the absorbance readings and assess the consistency of the analytical method (Yuliantini and Rahmawati, 2019).

E-ISSN : 2715-0518
P-ISSN : 2715-3053

6) Qualitative Sample Analysis

Up to 10 mL of the sample solution was carefully placed into a glass beaker using a pipette. Next, 1 mL of butterfly pea flower extract was added to the solution. The combination was then checked for color changes. If the resultant color matched or nearly resembled that of the positive control, the sample was considered Rhodamine B-positive.

Quantitative Analysis

1) Preparation of Rhodamine B Stock Solution

Rhodamine B dye weighed at 50 mg, is placed in a 50 ml measuring flask. Shake in enough methanol pro-analysis until homogenous. The solution is then filled with methanol pro-analysis up to the mark and homogenized. Dilution from stock solution was performed to make rhodamine B with a concentration of 50 mg/L. Pipette 2,5 mL of 1000 mg/L Rhodamine B solution, using a volume pipette, then put it into a 50 mL volumetric flask and add methanol pro-analysis up to the mark (Taupik, Mustapa and Gonibala, 2021).

2) Measurement of The Maximum Wavelength of Rhodamine B

Pipette using a volume pipette, transfer 2 ml of 50 mg/L Rhodamine B solution to a 50 ml volumetric flask. Add methanol pro-analysis to the mark and homogenize. Using a blank, measure the highest absorbance between 400 and 800 nm. The blank used is methanol pro-analysis (Taupik, Mustapa and Gonibala, 2021).

3) Linearity of Calibration Curves

In order to produce final concentrations of 2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L, 10 mg/L, and 12 mg/L, rhodamine B solution with a concentration of 50 mg/L was pipetted using a volumetric pipette and inserted successively into several 10 ml measuring flasks with volumes of 0.4 ml; 0.8 ml; 1.2 ml; 1.6 ml; 2 ml; and 2.4 ml. Each measuring flask was filled with methanol pro-analysis up to the line mark. The solution was then shaken to homogenize it. A UV-Vis Spectrophotometer was used to measure each solution's maximum absorbance at 545 nm (Taupik, Mustapa and Gonibala, 2021).

4) Quantitative Sample Analysis

The sample solution prepared according to the sample preparation procedure can then have its absorbance measured at a wavelength of 545 nm using a UV-Vis Spectrophotometer (Taupik, Mustapa and Gonibala, 2021).

III. RESULTS AND DISCUSSION

Plant species determination aims to authenticate the identity of the plant specimens to be researched while minimizing mistakes in the material collection and potential contamination with other plant species (Klau and Hesturini, 2021). The results of the test of plant determination at the Center for Information and Development of Traditional Medicine, University of Surabaya, revealed that the plant utilized was a butterfly pea plant with the Latin name (*Clitoria ternatea L.*) from the *Fabaceae* family.

E-ISSN : 2715-0518
P-ISSN : 2715-3053

Butterfly pea flower extraction was performed using the maceration method at room temperature with ethanol pro-analysis solvent for 24 hours (Yuliantini and Rahmawati, 2019). The method was selected because anthocyanins are stable at low temperatures (Dwiki, Nur and Fadraersada, 2018). Anthocyanins, which are polar molecules, need polar solvents for extraction, and ethanol pro-analysis was chosen for its low toxicity and excellent stability compared to other polar solvents (Yuliantini and Rahmawati, 2019). Maceration was carried out in a brown glass container to keep anthocyanins stable in the dark (Enaru *et al.*, 2021).

Following a 24-hour incubation period, the filtrate was separated using Whatman No. 1 filter paper and the liquid extract of butterfly pea flowers was finally obtained. The resultant solution was stored in a brown glass container to ensure the stability and integrity of the anthocyanins present in the extract. This container was kept in a dark environment at room temperature to minimize exposure to light and temperature variations, which could potentially degrade the anthocyanins and affect the accuracy of subsequent analyses (Enaru et al., 2021; Amperawati et al., 2019).

The selectivity test in this research is a visual observation of butterfly pea flower extract that changes color after being introduced to a positive control, standard Rhodamine B, and a negative control, methanol pro-analysis. Visual observation reveals a color difference between the negative and positive controls. It can be seen in Figure 1 that the negative control (left) retains the blue hue associated with the butterfly pea flower extract, but the positive control (right) transforms from blue to purple.

Figure 1. Visual Observation Results

A UV-Vis Spectrophotometer was then used to check and discriminate the spectrum produced by the positive and negative controls, verifying color changes. The method underlying this quantitative test is the Lambert-Beer law, which explains that the amount of incoming light passing through an absorbent solution will increase with increasing thickness of a solution concentration and decrease with decreasing sample transmittance. Figure 2 shows that the wavelength of Rhodamine B is not readable in the negative control (a), but in the positive control containing 10 mg/L Rhodamine B, an absorption of 0.6290 is read at a wavelength of 545 nm (b). Taupik et al. (2021) report a wavelength of 545 nm with an absorption of 0.2260 for the positive control. The selectivity test demonstrates that the butterfly pea flower extract can differentiate solutions containing Rhodamine B.

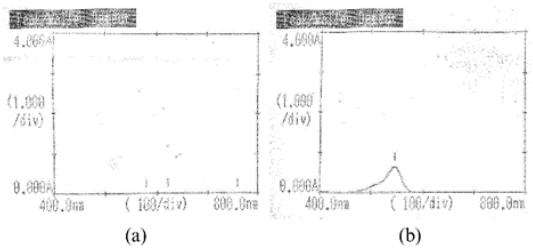

Figure 2. Negative Control Spectrum (a) and Positive Control Spectrum (b)

E-ISSN : 2715-0518
P-ISSN : 2715-3053

The next method validation step is to resolve the detection limit by serial dilution. Standard Rhodamine B solutions at various concentrations (1, 5, 10, 25, 50, 100, 250, 500 mg/L) were reacted with butterfly pea flower extract, as seen in the color series in Figure 3.

Figure 3. The Color Change of Butterfly Pea Flower Extract

The butterfly pea flower extract turns pink instead of blue in the image, suggesting that the solution is getting more acidic as the Rhodamine B concentration rises (to the right). Choriayah (2019) and Fauzi et al. (2022) discovered that the pH of Rhodamine B varies depending on the acid-base conditions of the solvent. Because methanol is a weak acid, Rhodamine B interacting with butterfly pea blossoms will produce an acidic environment.

The equilibrium reaction in the anthocyanin content of the butterfly pea flower itself causes the colour change of the butterfly pea flower extract. Anthocyanins in solution form are divided into five equilibrium forms, where these five forms depend on the pH level. The five forms are flavilium cation, carbinol base, chalcone, quinonoidal base, and quinonoidal anionic. Figure 4 shows the mechanism of the anthocyanin form change.

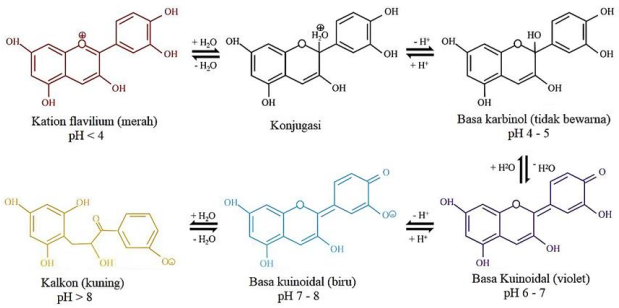

Figure 4. Anthocyanin Form in Equilibrium
(Source : Ifadah *et al.* (2021))

Visual observation revealed that the detection limit was at the Rhodamine B 25 mg/L standard, as it began to display a colour difference with the negative control, which was first blue to reddish purple. Furthermore, an accuracy test was performed by measuring the absorbance of the Rhodamine B 25 mg/L solution at 545 nm six times, yielding a relative standard deviation (%RSD) result. The precision test results are provided in Table 1.

Table 1. Precision Test Results

Replication	Absorbance	Average	SD	%RSD
1	1,5636	1,5663	0,00278	0,18%
2	1,5642			
3	1,5638			
4	1,5660			
5	1,5697			
6	1,5704			

The precision test findings indicate that the butterfly pea flower extract technique has high repeatability, with a %RSD value of 0.18%, meeting the $\leqslant$ 2% standard (Kamilla, Ramadhanty and Purwaningsih, 2020). A lower RSD % number suggests that more accuracy is preferable when testing a chemical molecule (Rohmah, Muadifah and Martha, 2021).

E-ISSN : 2715-0518
P-ISSN : 2715-3053

After being deemed legitimate, five eyeshadow samples from various brands will be examined qualitatively. As seen in Figure 5, all butterfly pea flower extracts change colour when interacting with the sample. Colour changes vary according to the preceding colour series, specifically shifts from purple to pink.

Figure 5. Qualitative Analysis Results

The quantitative analysis was then performed using a visible spectrophotometer. It began by finding the maximum wavelength in the 400-800 nm range using a working standard of 2 mg/L, resulting in the standard wavelength of Rhodamine B of 545 nm. The absorbance of the standard Rhodamine B solution was then tested at concentrations of 2, 4, 6, 8, 10, and 12 mg/L, with the findings shown in Table 2.

Based on the research's results, it is clear that the absorbance of the rhodamin b working standard meets the BPOM standards for excellent absorbance, which ranges from 0.2 to 0.8 (Ahriani *et al.*, 2021).

Table 2. Linearity Test Results

Concentration (mg/L)	Absorbance
2	0.1373
4	0.2771
6	0.4215
8	0.5906
10	0.7318
12	0.8661

Furthermore, the standard curve regression equation can be calculated using this data, yielding the equation y = 0.074x - 0.0137 with a r^2 value of 0.9991, as shown in Figure 6. The correlation coefficient (r) may be determined using the given r^2 value as 0.99955. Given that SNI defines a good linearity test as having a correlation coefficient (r) value of 0.995, it is reasonable to assume that the correlation coefficient (r) value meets the standards of an excellent linearity test (Kurniawan *et al.*, 2023).

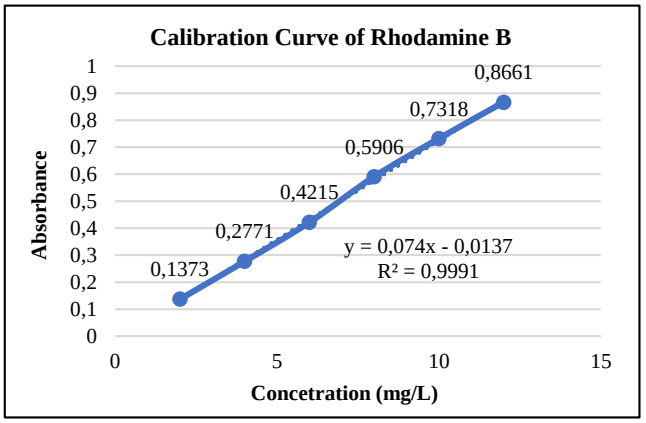

Figure 6. Calibration Curve of Rhodamin B

Since the regression equation has been obtained, the concentration of each blusher sample may be determined. The previously produced samples were tested for absorbance at 545 nm and repeated three times. The absorption and concentration of rhodamine b were determined by measuring the blusher sample using a visible spectrophotometer, as shown in Table 3.

Table 3. Quantitative Analysis Results

Number	Replication	Absorbance	Concentrarion (mg/L)	Average (mg/L)
1	1	0.2187	3.141	
1	2	0.2044	2.948	3.052
1	3	0.2132	3.066	
2	1	0.0161	0.403	
2	2	0.0196	0.450	0.419
2	3	0.0163	0.405	
3	1	0.6559	9.049	8.943
3	2	0.6486	8.950	

E-ISSN : 2715-0518
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3	3	0.6397	8.830	
4	1	1.1640	15.915	
4	2	1.1439	15.643	15.891
4	3	1.1789	16.116	
5	1	0.7385	10.165	
5	2	0.7291	10.038	10.090
5	3	0.7312	10.066	

IV. CONCLUSION

The color change of the butterfly pea flower extract, which came from blue to purple to pink after reacting with the sample, indicated that all five eyeshadow samples tested positive for containing the dangerous substance rhodamine B, according to the findings of the qualitative analysis. This indicates also that Rhodamine B can be found in cosmetic products using the butterfly pea flower ethanol extract. The hazardous substance Rhodamine B was then found in all five samples at varying concentrations (0.419 mg/L to 15.891 mg/L) based on the results of the quantitative analysis. This is in contrast to the BPOM's recommendation that cosmetic preparations not contain any Rhodamine B at all, or have a concentration 0 mg/L.

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