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Determination of Hydroquinone and Retinoic Acid in Whitening Creams in Ujung Berung Market Bandung, Using UV-Visible Spectrophotometry

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ABSTRACT

Whitening creams are cosmetic products applied to the skin to brighten or alter skin tone. The use of retinoic acid and hydroquinone without medical supervision is prohibited under Indonesian Food an Drug Authority (BPOM) regulations, as these are classified as prescription drugs that may cause harmful side effects with long-term use, such as skin irritation, dryness, burning sensation, and teratogenic effects. Unfortunately, these substances are still misused in some facial whitening products. This study aimed to identify the presence and determine the concentration of retinoic acid and hydroquinone in facial whitening creams sold in Ujung Berung Market, Bandung City. Samples were selected using purposive sampling with the criteria of being low-cost and without a registration number. A total of four cream samples were analyzed qualitatively using Thin Layer Chromatography (TLC) with a mobile phase of acetone:n-hexane (4:6), and quantitatively using UV-Visible spectrophotometry. Absorbance measurements for retinoic acid and hydroquinone were performed at wavelengths of 324 nm and 300 nm, respectively. TLC results showed that two samples, B and D, tested positive for retinoic acid with Rf values of 0.52 and 0.55, respectively, exhibited dark blue spots similar to the standard. The result of hydroquinone analysis using TLC was unidentified. Quantitative analysis revealed that sample B contained the highest retinoic acid concentration (0.077% w/w), while sample A had the highest hydroquinone concentration (0.604% w/w). These findings suggest that some facial whitening creams sold in Ujung Berung Market still contain unauthorized levels of retinoic acid and hydroquinone, highlighting the need for stricter regulation and public awareness.

Keywords: *Hydroquinone, Retinoic Acid, Thin Layer Chromatography (KLT), UV-Vis Spectrophotometry, Whitening Cream.*

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1. Introduction

The word "cosmetic" comes from the Greek term *kosmetikos*, meaning the art of beautifying. According to Indonesia's BPOM Regulation No. 23 of 2019, cosmetics are products applied to the outer parts of the body—such as the skin, hair, nails, lips, and teeth—with the main purpose of cleaning, beautifying, adding fragrance, protecting, or maintaining overall hygiene(1,2). One of the popular cosmetic products in Asia and Indonesia is skin whitening cream. These creams are used to lighten skin tone and reduce the appearance of dark spots or uneven pigmentation. However, many of these products contain harmful substances, such as hydroquinone, which is known for its potential side effects including skin irritation, ochronosis, and long-term health risks. Despite well-documented health risks, skin whitening creams remain widely used, largely driven by prevailing beauty ideals that associate lighter skin with attractiveness (3). Among the common ingredients found in these products are hydroquinone and retinoic acid—both of which are banned from use in cosmetics. While retinoic acid is known for its anti-aging benefits, such as improving skin texture and reducing wrinkles, its use is prohibited due to serious health concerns, including skin irritation and teratogenic risks(4,5).

Altough regulatory bodies like Indonesia's BPOM have prohibited the use of hydroquinone and retinoic acid in cosmetic formulations, multiple studies have shown that these substances are still commonly found in facial whitening creams sold in Indonesia. These products have been detected in samples obtained from traditional markets(6–8), beauty salons, and minimarkets across different regions(9,10). Such findings highlight an ongoing issue with the illegal inclusion of hazardous chemicals in cosmetic products, raising significant public health concerns. To detect and quantify these compounds, analytical techniques such as Thin Layer Chromatography (TLC)(11), UV-Visible spectrophotometry(12), and High-Performance Liquid Chromatography (HPLC)(13) are frequently utilized due to their effectiveness and reliability. Based on the findings of previous studies, the authors investigated on facial whitening creams available in the traditional market of Ujung Berung, West Java, to assess the presence and levels of hydroquinone and retinoic acid in these products.

2. Method

2.1 Materials and equipment

The chemicals and reagents used included acetone p.a (Merck, C_3H_6O), n-hexane p.a (Merck, C_6H_{14}), retinoic acid standard (Sigma, $C_{20}H_{28}O_2$), hydroquinone standard (Merck, $C_6H_6O_2$), methanol p.a (Merck, CH_3OH), ethanol 96%, distilled water (aquadest), silica gel 60 F254 TLC plate. The equipment utilized in this study consists of a TLC chamber, Whatman No. 41 filter paper, volumetric flasks, a UV lamp at 254 nm, a water bath, capillary tubes, volumetric pipettes, UV-Visible spectrophotometer (DLAB SP-V1100), analytical balance, centrifuge tubes, vortex and other glasswear obtained from Laboratory of Chemistry, Universitas Muhammadiyah Bandung.

2.2 Sample Collection and Organoleptic Examination

The samples were whitening creams sold at Ujung Berung traditional market in Bandung City, a location known for its popularity among local consumers. The sampling

conducted with purposive sampling. Four different samples of whitening creams were selected based on criteria: products without a BPOM registration number and with low prices (ranging from IDR 15,000 to 40,000). Exclusion criteria included products with well-known brands and higher prices (above IDR 50,000).

2.3 Qualitative Analysis Using Thin Layer Chromatography (TLC)

2.3.1 TLC Analysis of Retinoic acid

The mobile phase was prepared by mixing n-hexane and acetone in a 6:4 ratio, using a total volume of 2 mL. A standard solution was made by dissolving 0.01 g of retinoic acid in a 10 mL volumetric flask and diluting to volume with methanol. The sample and standard solutions were spotted onto the plate using capillary tubes. The plate was placed in a chromatography chamber pre-saturated with the mobile phase. Once the solvent front approached the upper limit, the plate was removed, dried, and observed under UV light at 254 nm. A positive result was indicated by the presence of a dark blue spot in the sample that matched the Rf value of the retinoic acid reference(14).

2.3.2 TLC Analysis of Hydroquinone

The thin-layer chromatography (TLC) procedure for hydroquinone was carried out using a mobile phase of n-hexane: acetone (6:4). A standard solution was prepared by accurately weighing 0,02 g of hydroquinone standard, dissolving it in 5 mL of 96% ethanol, and diluting to 10 mL with the same solvent. For the sample solution, approximately 1.5 g of whitening cream was weighed, mixed gradually with 15 mL of 96% ethanol, transferred to a 25 mL volumetric flask, and homogenized using an ultrasonic bath for 10 minutes. After cooling to room temperature, the solution was diluted to volume, chilled in an ice bath for fat separation, and filtered. The sample and standard solutions were spotted onto the plate using capillary tubes. Once the solvent front approached the upper limit, the plate was removed, dried, and observed under UV light at 254 nm, calculate the Rf value and compare it with the reference(14).

2.4 Quantitative Analysis of Retinoic Acid Using UV-Visible Spectrophotometry

2.4.1 Preparation of Retinoic Acid Standard Solutions

A stock solution of 1000 ppm was prepared by dissolving 0,1 g of retinoic acid in 100 mL methanol. Then 100 ppm solution was made by further dilution. To determine the maximum absorption wavelength, a 6 ppm diluted solution was scanned between 300–400 nm. Furthermore, the calibration curve and linearity were established using serial dilutions of 2, 4, 6, 8, and 10 ppm(15,16).

2.4.2 Sample Preparation and Retinoic Acid Analysis

Three grams of each cream sample were weighed and placed in a beaker, wrapped in aluminum foil. Ten milliliters of methanol were added, and the mixture was shaken until homogeneous. The solution was then cooled in an ice bath for 15 minutes and filtered using Whatman No. 41 filter paper. The filtrate was collected in a 50 mL volumetric flask and diluted to volume with methanol. From this solution, 5 mL was pipetted and transferred to a 10 mL volumetric flask, diluted again to the mark with methanol, and homogenized. The final solution was analyzed using UV-Visible spectrophotometry, measuring absorbance at the previously determined maximum wavelength(16).

2.5 Quantitative Analysis of Hydroquinone Using UV-Visible Spectrophotometry

2.5.1 Preparation of Hydroquinone Standard Solutions

A stock solution of hydroquinone was prepared by dissolving 5 mg of hydroquinone standard in methanol, then transferring it to a 100 mL volumetric flask and diluting to volume with methanol to obtain a concentration of 50 ppm. A maximum wavelength was determined from a 20 ppm hydroquinone scanned between 200–400 nm. A standard calibration curve was created by diluting this stock solution to concentrations of 6, 8, 10, 12, and 16 ppm and was measured using a UV-Visible spectrophotometer at the maximum wavelength(17). From the calibration curve equation, the coefficient of determination (r^2) was calculated to assess the linearity of the method. In addition, the Limit of Detection (LoD) and Limit of Quantification (LoQ) were determined to evaluate the sensitivity of the method.

2.5.2 Sample Preparation and Hydroquinone Analysis

Weight 25 mg of each whitening cream was suspended in 50 mL ethanol, heated, homogenized, and filtered. The hydroquinone content was then quantified using UV-Vis spectrophotometry at 300 nm, and concentrations were calculated using the linear regression equation from the calibration curve (18).

3. Result

3.1 Organoleptic Examination result

The characteristics of the whitening cream samples were initially evaluated through organoleptic testing, focusing on color, odor, and texture, as presented in Table 1.

Table 1. Organoleptic of Whitening Cream Sampels

Sample	Color	Odor	Texture
A	Bright yellow	Strong scent and acidic	Soft and sticky
B	Pale yellow	Strong scent	Soft and very sticky
C	Pale yellow	Strong scent and acidic	Soft and sticky
D	Pale white	Fragrant	Soft, slightly oily

3.2 Thin Layer Chromatography (TLC) of Retinoic Acid Result

The results of the Rf value determination and spot color of retinoic acid by TLC, as shown in Table 2, indicate that three samples B, C, and D were identified as positive.

Table 2. Rf Values for Retinoic Acid Reference and Samples

Sample	Rf	Spot Color	Result
Reference	0.55	Dark blue	Positive
A	-	-	Negative
B	0.525	Dark blue	Positive
C	0.075	Dark blue	Negative
D	0.55	Dark blue	Positive

Positive (+) if the Rf value differs by ±0.05 from the reference.
Negative (−) if the Rf value differs by more than 0.05 from reference.

3.3 Thin Layer Chromatography (TLC) of Hydroquinone

The results of TLC spot observation and Rf value calculation for hydroquinone reference and samples, as shown in Table 3. Its indicate that none of the samples tested positive for hydroquinone.

Table 3. Rf Values for Hydroquinone Reference and Samples

Sample	Rf Value	Spot Color	Result
Reference	0.175	Dark Blue	Positive
A	-	-	Negative
B	-	-	Negative
C	-	-	Negative
D	0.45	Dark Blue	Negative

Positive (+) if the Rf value differs by ±0.05 from reference
Negative (−) if the Rf value differs by more than 0.05 from the reference.

3.4 Quantitative Analysis of Retinoic Acid Using UV-Visible Spectrophotometry

The maximum wavelength of retinoic acid was measured within the range of 300–400 nm, with the peak absorbance observed at 324 nm and an absorbance value of 0.68. The calibration curve is shown in Figure 1, with a regression equation of y = 0.071x + 0.1118 and a determination coefficient (r^2) of 0.9886, indicating good linearity. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated to be 1.0187 ppm and 3.395 ppm, respectively.

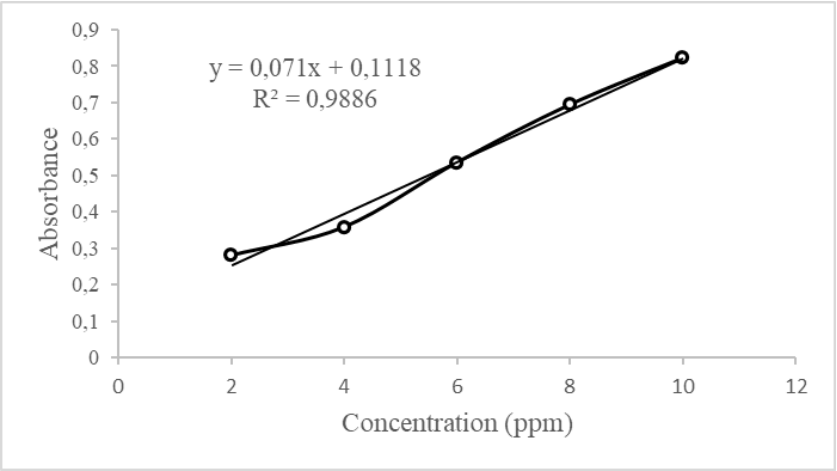

Figure 1. Calibration curve of Ratinoic acid reference

The results of the quantitative analysis for retinoic acid in the whitening cream samples are summarized in Table 3. The average concentrations of retinoic acid were found to be 0.063% w/w in Sample A, 0.338% w/w in Sample B, 0.050% w/w in Sample C, and 0.077% w/w in Sample D.

Table 3. Content of Retinoic acid (w/w%) in Whitening Creams

Sample	Absorbance	Content (w/w%)
A	0.382	0.063
B	1.555	0.338
C	0.325	0.050
D	0.444	0.077

3.5 Quantitative Analysis of Hydroquinone Using UV-Visible Spectrophotometry

The maximum wavelength of hydroquinone was determined within the range of 200–400 nm the result show maximum wavelength observed at 300 nm with absorbance 0.541. The calibration curve is shown in Figure 2 and give the linear regression equation of y = 0.0457x + 0.0996, with a correlation (r^2) of 0.9987. The calculated Limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.4235 ppm and 1.4118 ppm, respectively.

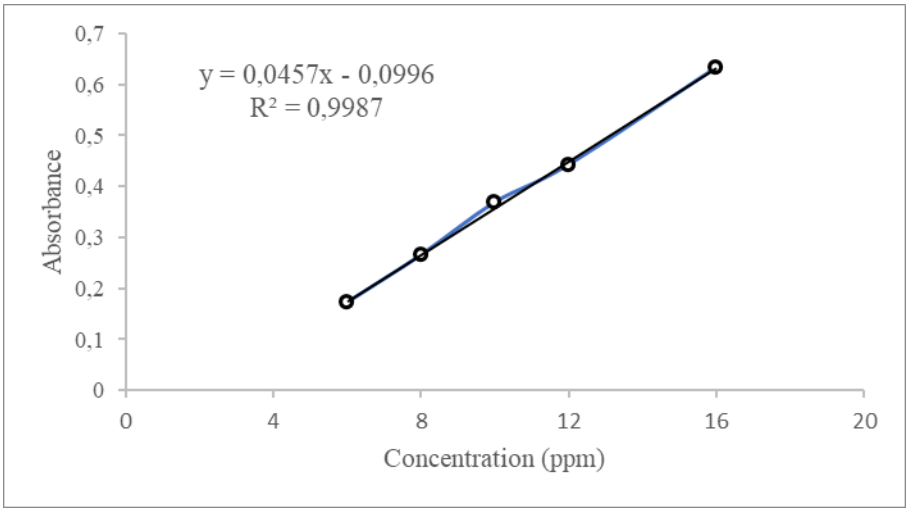

Figure 2. Calibration curve of Hydroquinone reference

According to the results presented in Table 4, the average concentrations of hydroquinone in the facial whitening cream samples were found to be 0.604% w/w in Sample A, 0.555% w/w in Sample B, 0.576% w/w in Sample C, and 0.495% w/w in Sample D.

Table 4. Content of Hydroqunone (w/w%) in Whitening Creams

Sample	Absorbance	Content (w/w%)
A	0.841	0.604
B	0.596	0.555
C	0.702	0.576
D	0.297	0.495

4. Discussion

4.1 Organoleptic of Whitening Cream Sampels

The organoleptic examination revealed that all whitening creams samples generally have a sticky texture, a sour and strong scent. These characteristics align with the typical characterictic of creams containing retinoic acid, which are often pale yellow to white in color, sticky in texture, and emit a strong and sour odor(19). The presence of a sticky texture in a cream may indicate the inclusion of harmful substances, as it suggests the presence of compounds—such as heavy metals—that have strong binding properties and can interact with surrounding ions. Furthermore, the intense odor in the creams may also serve as an indicator of harmful ingredients. In many cases, such strong scents are

the result of excessive use of essential oil-based perfumes added to mask the unpleasant scent of hazardous components within the formulation(20).

4.2 Qualitative Analysis of Retinoic acid and Hydroquinone using TLC

The mobile fase acetone: n-hexan (4:6) was chosen because the solvent mixture provided effective separation and complied with BPOM guidelines(14). The mobile phase is non-polar, while the stationary phase—silica gel GF 254—is polar. Since the sample tends to be polar as well, the standard solution and the sample can be separated due to their differing polarity properties. Based on the results of the TLC test, the Rf values observed for samples B (0,525) and D (0,55) were similar to that of the retinoic acid standard, which had an Rf value of 0,55 with a dark blue spot. The difference in Rf values between the standard and samples B and D was only 0,025. A sample is considered positive for retinoic acid if the Rf difference with the reference standard is ≤ 0,050(21). Among the four unregistered whitening cream samples tested, two—samples B and D—were identified as containing retinoic acid. Both samples produced dark blue spots that resembled those of the retinoic acid standard.

Based on the TLC analysis, the reference hydroquinone showed an Rf value of 0,175 with a dark blue spot. The analyzed samples—A, B, C, and D—did not produce spots similar to that of hydroquinone, indicating that they are likely negative for hydroquinone content. Thin Layer Chromatography (TLC) is widely used for the qualitative analysis of hydroquinone in cosmetic products due to its simplicity and cost-effectiveness. However, the specificity of TLC can be influenced by the presence of other compounds that may produce similar Rf values or spot colors, potentially leading to false positives or negatives. Therefore, while TLC serves as a useful preliminary screening method, its findings should be confirmed using more sensitive and specific techniques such as UV-Vis spectrophotometry or High-Performance Liquid Chromatography (HPLC) (22,23)

4.3 Quantitative Analysis of Retinoic acid using Spectrophotometry UV-Vis

According to the analysis results presented in Table 4, although samples A, C, and D fall within the previously accepted therapeutic topical range of 0.05–0.1%, Indonesia's National Agency of Drug and Food Control (BPOM) currently prohibits the use of retinoic acid in cosmetic products regardless of concentration. This regulation, first enforced in 1998 and reaffirmed in recent updates, is based on the potential for skin irritation and systemic side effects, particularly when the ingredient is used without medical supervision (1). In contrast, sample B contained a significantly higher concentration of retinoic acid at 0.338% w/w, which not only violates the regulatory limits but also poses a greater health risk. This finding indicates that sample B is non-compliant with BPOM regulations, as retinoic acid is classified as a prescription-only active pharmaceutical ingredient and should only be used under a physician's supervision.

Several other studies in Indonesia have also reported similar findings regarding the presence of retinoic acid in illegal cosmetic products. For example, retinoic acid found in whitening creams sold in traditional markets in Semarang ranging from 0,16% to 0,20% w/w concentrations(24). Additionally, night

cream products in Pekalongan detected retinoic acid with levels between 0,08% and 0,09% w/w also surpassing safety thresholds(25). These findings indicate that many unregistered cosmetic products containing harmful substances are still circulating in traditional markets in Indonesia.

The method used demonstrated a high degree of linearity, with a correlation coefficient (r) of 0.9886, which is close to the ideal value of 1.0, and complies with the analytical method validation requirements outlined in the ICH Q2(R1) guidelines(26). The LOD and LOQ obtained indicating that this method is sufficiently sensitive for detecting the analyte at low concentrations compared to multile previous studies employing UV-Vis spectrophotometric methods for the analysis of retinoic acid (27).

4.4 Quantitative Analysis of Hydroquinone using Spectrophotometry UV-Vis

The determination of hydroquinone concentration using UV-Vis spectrophotometry revealed levels ranging from 0.495% to 0.604% w/w. These findings suggest that thin-layer chromatography (TLC) lacks sufficient sensitivity to detect hydroquinone at low concentrations. Furthermore, the presence of hydroquinone in these whitening creams—none of which were registered with the Indonesian National Agency for Drug and Food Control (BPOM)—indicates that such products are illegally distributed and should not be available on the market. According to BPOM Regulation No. 23 of 2019, the use of hydroquinone in cosmetic products is prohibited due to its potential to cause skin irritation, ochronosis (a type of skin hyperpigmentation), and possible carcinogenic effects with prolonged use(1,28).

Comparable results were reported in other studies conducted in traditional markets across Indonesia such as in Segiri Market, Samarinda and Jayapura was identified various levels of hydroquinone in whitening creams(20,29). These results reinforce concerns regarding the continued circulation of unregulated and potentially harmful cosmetic products containing hydroquinone in various regions of Indonesia.

The UV-Vis spectrophotometric method applied in this study exhibited good linearity, with a correlation coefficient of 0.9987, indicating a strong linear relationship between analyte concentration and absorbance (26). The method also demonstrated adequate sensitivity, as shown by the LOD and LOQ values of 0.4235 ppm and 1.4118 ppm, respectively. Compared to previous studies reporting linearity, LOD and LOQ values range, the current method falls within acceptable and reliable limits(27). These results confirm that the method is linier and sensitive for the determination of hydroquinone in cosmetic products.

5. Conclusion

Qualitative and quantitative analyses of retinoic acid and hydroquinone in whitening creams sold at Ujung Berung Market, Bandung, have been conducted. Thin-layer chromatography (TLC) results indicated the presence of retinoic acid in three samples, while hydroquinone was not detected using the same method. However, further analysis using UV-Vis spectrophotometric analysis revealed that samples A, B, C, and D contained both retinoic acid and hydroquinone at varying concentrations. These findings indicate that several whitening cream products circulating in the market do not comply with BPOM regulations, highlighting the need for increased awareness and caution in selecting facial whitening products.

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