Application of Bixin Extract from *Bixa orellana L.* Seeds as a Natural Colorant in Compact Powder Eyeshadow Formulation

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Abstract. Annatto seeds (*Bixa orellana L.*) contain natural pigments, namely bixin and norbixin. Bixin can produce a reddish-orange color, commonly used as a natural colorant in food, textiles, and cosmetics. However, annatto seeds are still never used in compact powder eyeshadow formulations. This research aimed to produce bixin extract from *Bixa Orellana L.* through extraction and vacuum evaporation processes, and to determine the optimal bixin extract as a natural colorant in compact powder eyeshadow formulation. The extract was obtained by an extraction process using ethyl acetate as a solvent. The extraction process was done in a stirred extractor at 50°C and 300 rpm for 90 minutes. The extract was then concentrated using a rotary vacuum evaporator at 35°C, followed by centrifugation for 10 minutes. The result of the bixin extracted from annatto seeds was 10.48 mg bixin/gram, while the yield was 1.2%. The compact powder eyeshadow formulations were prepared at bixin extract concentrations: 10% (F1), 20% (F2), and 30% (F3). The evaluation of the compact powder eyeshadow formulations included organoleptic, homogeneity, spreadability, irritation, and hedonic tests. The result was the F2 formula, which indicated the most optimal formulation based on the evaluation conducted.

1 Introduction

Herbal or natural-based cosmetics are now a trend in the fashion and beauty industry. Cosmetics are products used on the skin to cleanse, beautify, or change appearance, and protect, but not to treat disease [1]. One of the components of cosmetics is coloring agents. However, many cosmetic products on the market use synthetic colorants. Heavy metal contamination, such as Pb, Cd, Hg, As, and Cr, is often found in synthetic colorants in cosmetics, which can cause carcinogenic, allergenic, and neurotoxic effects [2]. Natural colorants offer an alternative to synthetic colorants since they are non-toxic, non-carcinogenic, biodegradable, and environmentally friendly [3].

One of the cosmetic products that women often use is eyeshadow. Eyeshadow is a beauty product made specifically to give color and beautify the eyelid area, especially the upper part of the eyelid. Eyeshadow is usually in compact powder, but also available in other forms, such as loose powder, cream, stick, and liquid [4]. As a cream, cream eyeshadow can be applied by rubbing your fingertips on the eyelids. Cream eyeshadow is more moisturizing

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than compact powder eyeshadow because it uses a higher oil content as its base. However, because it contains volatile binding agents, emulsifiers, and surfactants, cream eyeshadow can cause skin irritation [5]. Compact powder eyeshadow is a dry powder that has been compressed into a compact form, which can be applied using a brush or puff. It has good adhesion, can absorb oil, and provides a soft effect when used [6].

Bixa orellana L. is one of the plants that has high potential as a colorant. Annatto obtained from *Bixa orellana L.* fruit is one of the natural colorants frequently found in food products, cosmetics, and pharmaceuticals because it has optimal color intensity and good stability [7]. The main pigments of annatto are carotenoids consisting of bixin and norbixin [8, 9]. Bixin is the basic color pigment of annatto and is naturally found in a thin resin layer that coats the non-polar seeds. Although bixin is nonpolar, it is also soluble in many polar compounds, like ethanol, methanol, acetone, and ethyl acetate. [7,8]. Annatto contains four to nine times more carotenoids than carrots, tomatoes, and corn, of which 80% is bixin [10]. Bixin is responsible for imparting reddishness, and norbixin is responsible for imparting yellow [11].

In recent years, research related to the manufacture of cosmetics using natural colorants continued to develop, including its use in compact powder eyeshadow formulations [12]. Previous studies have examined the use of various natural ingredients as compact powder eyeshadow colorants, such as butterfly pea flower extract (*Clitoria ternatea)* [13], purple sweet potato extract (*Ipomoea batatas L.*) [14], *Syzygium cumini* fruit extract [15], red spinach leaf extract (*Amaranthus tricolor L.*), and miana leaf extract (*Coleus scutellarioides L.*). Using *Bixa orellana L.* as a natural colorant in compact powder eyeshadow formulation is an opportunity for novelty in this research. Previously, *Bixa orellana L.* has been researched to determine the optimal solvent in the extraction process to be used in lip gloss products. Some solvents commonly used in bixin extraction are acetone, ethanol, and ethyl acetate. The most effective order of solvents for dissolving bixin is acetone, ethyl acetate, and ethanol [12]. In this study, the solvent used was ethyl acetate. Ethyl acetate was chosen over acetone because ethyl acetate has been declared safe for extracting active ingredients from natural materials by the Indonesian Food and Drug Administration (BPOM), while acetone has not.

This research aimed to produce bixin extract from *Bixa orellana L.* through extraction and vacuum evaporation processes, and to determine the optimal bixin extract as a natural colorant in compact powder eyeshadow formulation. The goal was to make sure the compact powder eyeshadow meets the specified criteria, enhance the quality of cosmetics, especially for women, by enhancing appearance or beauty, and reduce any potential risks for its users.

Bixin can be obtained by an extraction process where a solvent is utilized to extract several substances from solids or liquids. The bixin extraction process can be done by the maceration method, which involves soaking annatto *(Bixa orellana L.)* seeds in a solvent. In addition, other extraction methods to improve effectiveness have been developed recently, such as microwave-assisted extraction [7], ultrasonic-assisted extraction [9], the stirred extractor [12], the Soxhlet and reflux method, and supercritical fluid extraction. In this study, the extraction method was carried out using a stirred extractor with ethyl acetate as the solvent. After extraction, solvent evaporation was carried out using a rotary vacuum evaporator. The concentrated extract was centrifuged to obtain an extract precipitate, which was then formulated into a compact powder eyeshadow formulation. Various tests were carried out on the preparation, including irritation, homogeneity, organoleptic, spreadability, and hedonic tests.

2 Research and Methodology

2.1 Materials

The materials used in colorant extraction were annatto seeds (from Bantul, Yogyakarta) and solvent, ethyl acetate (99.5%, Fulltime, China). The material used to measure bixin content was chloroform (99%, Merck, Germany). The ingredients used to produce the compact powder eyeshadow included zinc stearate (Bo Young Chemical, Korea), isopropyl myristate (Brataco, Indonesia), phenoxyethanol (Brataco, Indonesia), and talc (Haichen Talc Powder, Tangerang).

2.2 Extraction and Vacuum Evaporation of Annatto Seeds

Fresh annatto seeds were obtained from Bantul, Yogyakarta. Extraction was carried out by a stirrer-equipped extractor. Annatto seeds were soaked in ethyl acetate solvent, with each 100 grams of annatto seeds soaked in 1000 mL of solvent (1:10 w/v). A stirrer was used to perform the extraction for 90 minutes at 50°C and 300 rpm. After extraction, a rotating vacuum evaporator was used to concentrate the extract at 35°C. After that, centrifugation was performed for 10 minutes at a rotational speed of 15 rpm to obtain a precipitate. The resulting bixin extract had a dark reddish hue.

The bixin content in annatto extract was analyzed using a UV-Vis spectrophotometer. A total of 0.1 mL of the extract sample was dissolved using chloroform to a volume of 100 mL. The absorbance spectrum was obtained within the 400–600 nm range using a 1 cm path-length cuvette. The wavelength corresponding to the maximum absorbance (λmax) was identified at approximately 498 nm for the extract with ethyl acetate as a solvent. Absorbance readings were also taken at 404 nm. Calculation of bixin content was carried out using the formula equation 1. Meanwhile, yield was calculated using the formula listed in equation 2.

$$Bixin\ content\ (\%) = \frac{[(Amax + A404) - (0.256A\mathrm{max})]}{282{,}6} \times \frac{100}{Sample\ (g)} \times \frac{Dilution\ Volume\ (ml)}{1000} \quad (1)$$

$$Yield\ (\%) = \frac{The\ precipitate\ of\ extract\ weight\ (g)}{Sample\ wight\ (g)} \times 100\% \quad (2)$$

2.3 Application of Bixin Extract in Compact Powder Eyeshadow Formulation

All components were prepared in accordance with the quantities stated in the formulation presented in Table 1, and the compact powder eyeshadow formulation was prepared. Zinc stearate and talc were sieved using a 100 mesh sieve and then spread slightly on a mortar. One by one, the ingredients (phenoxyethanol, isopropyl myristate) were added from the smallest to the largest amount while being crushed until homogeneous. After that, bixin extract was added as a colorant and mixed until homogeneous.

Table 1. Compact powder eyeshadow formulation

Material	Function	F1 (%)	F2 (%)	F3 (%)
Annatto seed extract	Color pigment	10	20	30
Phenoxyethanol	Preservatives	0,2	0,2	0,2
Zinc stearate	Adhesive, Anti-caking	10	10	10
Isopropyl Myristate	Binder	0,75	0,75	0,75
Talc	Filling material	Add 100	Add 100	Add 100

2.4 Evaluation of compact powder eyeshadow

All research activities were conducted in accordance with the provisions of Article 39 of the Indonesian Law No. 11/2019 regarding the National System of Science and Technology, which emphasizes the importance of adhering to ethical codes. Since human subjects were involved in the study, the research upheld the principles of participant protection and safety. Ethical approval has been granted by the Ethics Committee of dr. Moewardi Hospital, No. 1.730/VII/HREC/2025, enabling the research activities to proceed in accordance with regulations.

2.4.1 Organoleptic Test

The organoleptic test was a physical observation of compact powder eyeshadow preparations that have been formulated using sensory characteristics, such a scent, texture, and color tests.

2.4.2 Homogeneity Test

The homogeneity test was carried out by applying the formulation to the back of the hand. If the resulting color was consistent and there were no lumps, the formulation can be considered homogeneous.

2.4.3 Spreadability Test

The compact powder eyeshadow formulation was applied to the back of the hand for this test, and the pigmentation or color intensity that resulted was then observed.

2.4.4 pH Test

Compact powder eyeshadow was tested with a concentration of 1% by diluting 1 gram of the preparation sample in 100 mL of distilled water. After that, the pH indicator paper was dipped into the solution, and its color was matched with the existing pH scale. The appropriate pH range for skin cosmetic products was between 5 and 8.5.

2.4.5 Irritation Test

A total of twenty panelists were randomly selected to undergo irritation testing. The method used in the irritation test was a closed patch test on the inner upper arm. The skin was left for

2 days, then reactions were observed. Assessment of irritation was done based on the criteria listed in Table 2.

Table 2. Criteria for the irritation test

Score	Skin Reaction
0	No visible reaction
1	Redness
2	Itching sensation
3	Noticable swelling

2.4.6 Hedonic Test

The hedonic test was conducted to assess the level of preference of twenty randomly selected panelists towards each compact powder eyeshadow formulation. This favorability assessment refers to the criteria listed in Table 3. The hedonic test data were analyzed numerically using IBM SPSS (Statistical Package for the Social Sciences) Statistics software. The analysis began with a Kolmogorov–Smirnov test to determine whether the preference data for each compact powder eyeshadow formulation were normally distributed. If the significance value (p-value) was > 0.05, the data were considered to be normally distributed, and the analysis continued with an ANOVA (Analysis of Variance) test. Conversely, if the significance value was < 0.05, the data was considered to be non-normally distributed and a nonparametric Kruskal–Wallis test was used. The results of the Kruskal–Wallis test were reviewed through the Asymp. Sig. (Asymptotic Significance) value. If the Asymp. Sig. value was < 0.05, then there was a significant difference between formulations. To determine which formulations were significantly different, a post hoc test was performed using the Mann–Whitney test.

Table 3. Criteria for the hedonic test

Score	Description
9	Extremely Like
8	Really Like
7	Like
6	Enough Like
5	Neutral
4	Moderately Dislike
3	Dislikes
2	Strongly Dislike
1	Extremely Dislike

3 Results and discussion

3.1 Extraction and vacuum evaporation process

The extraction process of active compounds from annatto seeds was carried out using a stirred extractor with ethyl acetate as the solvent. Ethyl acetate was chosen based on the main properties of bixin (the main component of annatto seeds), which dissolve better in this solvent than in other solvents. The structure of bixin, which has a polyene chain as well as carboxylate and methyl ester groups, gives it a high affinity for semi-polar solvents. To get a high bixin content, the extraction process was conducted for 90 minutes at 50°C. Because of pigment degradation, extract concentrations might drop at temperatures higher than the solvent's boiling point. After the extraction process, the bixin content in annatto seed was analyzed using a UV-Vis spectrophotometer at a wavelength range of 400–600 nm. The calculation of bixin content followed the procedure used by Scotter (equation 1). The result of the bixin extracted from annatto seeds in this research was 10.48 mg bixin/gram. This is not much different from previous research that obtained a bixin content of 8.12 mg bixin/gram of annatto seeds [12].

To concentrate the extract, the extracted solution was then evaporated at 35°C using a rotary vacuum evaporator. Through vacuum evaporation, a total of 1000 milliliters of extract produced 45 milliliters of concentrated extract. After centrifugation, 1.2 grams of extract sediment weight were obtained from 100 grams of annatto seeds, resulting in a yield of 1.2%. It is higher than the yield of the other research that used the ultrasonic bath method at 30 °C for 60 minutes, with a yield of 0.602% [9]. These different yields are due to differences in extraction methods, temperatures, and times. In this study, extraction was carried out with stirring to increase contact between the solvent and the material. Meanwhile, the ultrasonic bath method uses ultrasonic waves to produce cavitation that can damage the cell structure of the material, resulting in the release of active compounds. Higher temperatures can optimize extraction because the solubility of bixin increases, but if the temperature is too high, it will damage the structure of bixin, causing the color to degrade.

3.2 Application and Evaluation of Bixin Extract in Compact Powder Eyeshadow Formulation

A compact powder eyeshadow formulation using *Bixa orellana L.* as a natural colorant was developed to create a variety of cosmetic options that can meet the diverse tastes of consumers. Each formulation had different visual characteristics, including color, texture, and softness. The use of natural pigments is expected not only to enhance aesthetic appeal but also to ensure safety in use.

The ethics committee of dr. Moewardi Hospital, Surakarta, has approved this study (No. 1.730/VII/HREC/2025), indicating that it has been evaluated by the relevant parties and complies with the ethical standards and principles required for its implementation. To assess product performance, the formulation results were compared with a commercial reference product (Brand X). The result of compact powder eyeshadow formulation are shown in Figure 1.

F1 F2 F3 Brand X

Fig. 1. Compact powder eyeshadow with 10% (F1), 20% (F2), 30% (F3) concentration of *Bixa orellana L.*, and Brand X

3.2.1 Organoleptic test

Organoleptic testing results for compact powder eyeshadow are presented in Table 4. Organoleptic testing was conducted based on sensory characteristics, such as scent, texture, and color.

Table 4. Compact powder eyeshadow organoleptic test

Formulation	Identification of Organoleptic Test			
	Preparation	**Color**	**Scent**	**Texture**
F1	Compact Powder	Yellowish	Fragrant	Stick Well
F2	Compact Powder	Reddish	Fragrant	Stick Well
F3	Compact Powder	Reddish	Fragrant	Stick Well

3.2.2 Homogeniety test

Homogeneity testing showed consistent results for compact powder eyeshadow formulation. When applied to transparent glass, the texture of all formulations was consistent. This indicates that the formulations were homogeneously mixed and well blended. The results of the homogeneity test are presented in Table 5.

Table 5. Result of the homogeneous test

Formulation	Homogeneous	Not Homogeneous
F1	√	
F2	√	
F3	√	

3.2.3 Spreadability test

To assess the ease of application and the resulting color clarity, a test of spreadability was carried out. Applying the compact powder eyeshadow on the back of the hand was necessary for this, with the results presented in Fig. 2.

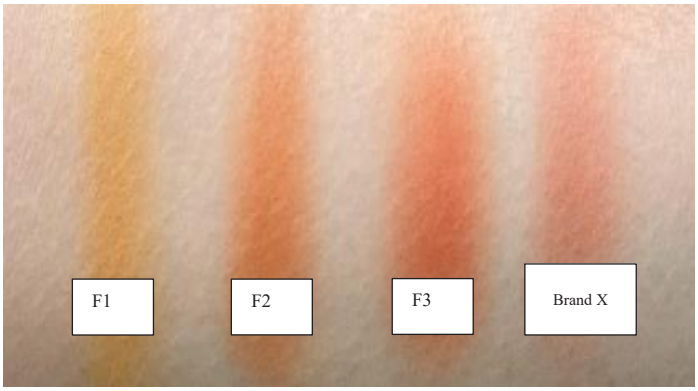

Fig. 2. Compact powder eyeshadow spread test results with 10% (F1), 20% (F2), and 30% (F3) bixin extract concentrations, and Brand X

3.2.4 pH test

The measurements of pH value, 1 gram of the preparation was diluted in 100 milliliters of distilled water to produce a 1% concentration of the sample. pH strips were used to determine the pH of the sample.

Table 6. Result of the pH test

Formulation	pH value
F1	5
F2	5
F3	5

pH value measured from the compact powder eye shadow formulation using *Bixa orellana L.* as a natural colorant is 5, which is within the safe pH range of 5-8.5. It is anticipated that an excessively acidic or alkaline pH of the preparation may irritate the skin. As a result, compact powder eyeshadow formulation compositions undergo pH tests in accordance with quality standards.

3.2.5 Irritation test

The irritation test was performed by leaving the skin exposed for two days, proving that this product was safe to use. The irritation test was conducted on the inner upper arm. Results obtained from the irritation test are presented in Table 7. From the data in Table 7, all of the scores of skin reaction were 0, which means no visible reaction (Table 2). The researchers did not find any irritation effects from the formulation, such as burning sensations, swelling, redness, or itching during the observation period involving twenty panelists. This indicates that there are no allergic reactions to the natural colorant of *Bixa orellana L.* used in the compact powder eyeshadow formulation. Therefore, the use of this mixture on human skin is considered safe.

Table 7. Result of the irritation test

Panelists	Skin Reaction						Panelists	Skin Reaction					
	First Day			Second day				First Day			Second Day		
	F1	F2	F3	F1	F2	F3		F1	F2	F3	F1	F2	F3
1	0	0	0	0	0	0	**11**	0	0	0	0	0	0
2	0	0	0	0	0	0	**12**	0	0	0	0	0	0
3	0	0	0	0	0	0	**13**	0	0	0	0	0	0
4	0	0	0	0	0	0	**14**	0	0	0	0	0	0
5	0	0	0	0	0	0	**15**	0	0	0	0	0	0
6	0	0	0	0	0	0	**16**	0	0	0	0	0	0
7	0	0	0	0	0	0	**17**	0	0	0	0	0	0
8	0	0	0	0	0	0	**18**	0	0	0	0	0	0
9	0	0	0	0	0	0	**19**	0	0	0	0	0	0
10	0	0	0	0	0	0	**20**	0	0	0	0	0	0

3.2.6 Hedonic test

Twenty randomly chosen panelists' acceptance and preference for the compact powder eyeshadow were evaluated using a hedonic test. After that, the panelists assigned ratings to the products according to how much they liked them. This test is important for comprehending customer satisfaction.

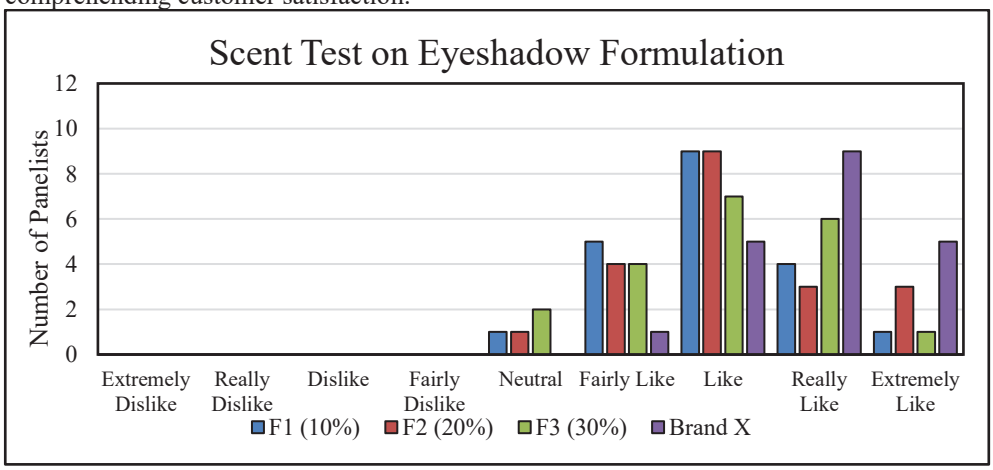

Fig. 3. Hedonic evaluation results for compact powder in a scent test

According to the scent parameter results of the hedonic test, 9 panelists gave formulations F1 and F2 a “like” rating, while 9 panelists gave Brand X a “really like” rating. In formulations F1, F2, and F3, no fragrances were added, but Brand X may have added a fragrance as one of its selling points. F3 was not as well-liked in terms of scent, likely due to the scent from *Bixa orellana L.* at the highest concentration.

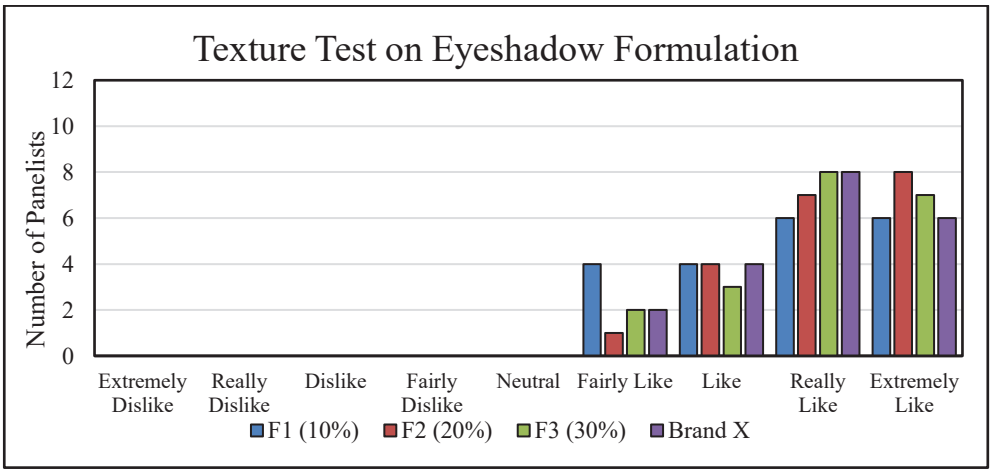

Fig. 4. Hedonic evaluation results for compact powder in a texture test

Figure 4 displays the outcomes of the hedonic test based on texture parameter. The most popular formulation is F2 (20%), as seen in Figure 4. The eyeshadow in F2 is considered smoother, easier to blend, and more comfortable to apply than the other formulations.

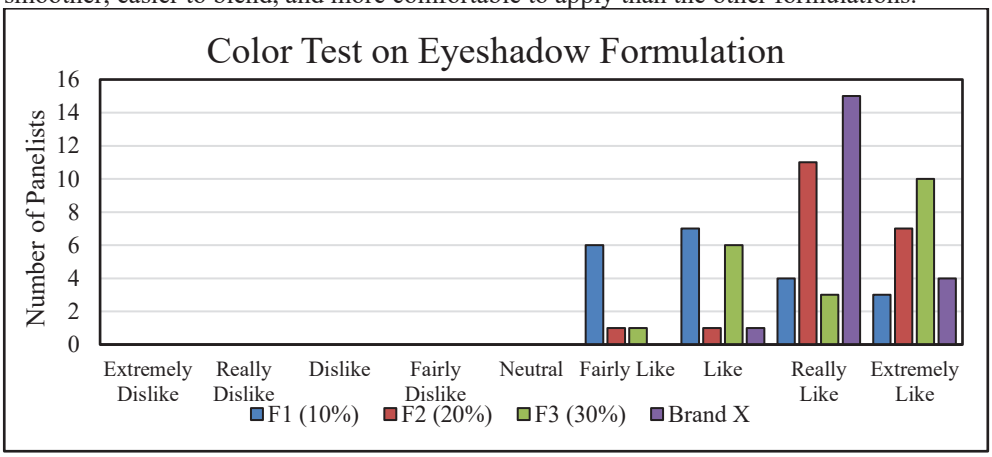

Fig. 5. Hedonic evaluation results for compact powder in a color test

All three formulations produced uniform colors according to color parameters. But the most popular formulation was F2 (20%), which 11 panelists rated as "really like" and 15 panelists rating brand X as “really like” too. Ten panelists also gave formulation F3 (30%) the rating of "extremely like." F1 produces a yellowish-orange color, whereas F2 and F3 both produce a reddish-orange color. The concentration of the colorant extract added to the formulation affects the color variations. Table 8 presents the results of statistical analysis obtained using SPSS software on the results of the hedonic test.

Table 8. The result of hedonic test

Parameter	The hedonic test average value			
	F1	**F2**	**F3**	**Brand X**
Scent	6.950 ± 0.945[a]	7.150 ± 1.089[a]	7.000 ± 1.076[a]	7.900 ± 0.852[b]
Texture	7.700 ± 1.129[a]	8.100 ± 0,912[a]	8.000 ± 0.973[a]	7.900 ± 0.968[a]
Color	7.200 ± 1.056[a]	8.200 ± 0.768[b]	8.100 ± 1.021[b]	8.150 ± 0.489[b]

[a, b:] Indicates that the Mann-Whitney test indicate significant differences.

The Kolmogorov-Smirnov test was used to test normality. According to the Kolmogorov-Smirnov normality test, the scent data are not normally distributed ($p < 0.05$). The Kruskal-Wallis test shows that the data are significantly different with a p-value of 0.012. Data are considered significantly different if the p-value is < 0.05. The Mann–Whitney test shows that scents of F1, F2, and F3 are not significantly different, but all three are significantly different from the scent of Brand X. The scent of Brand X is the most preferred scent (7.900 ± 0.852). Based on the preparation of F1, F2, and F3, the scent of F2 is the most preferred scent (7.150 ± 1.089).

Texture is the second parameter analyzed. The Kolmogorov-Smirnov normality test indicates that texture data is not normally distributed ($p < 0.05$). The texture data's p-value, as determined by the Kruskal-Wallis test, was 0.700. This value confirms that the textures of F1, F2, F3, and Brand X are not significantly different because $p > 0.05$. Although not significantly different, according to the panelists, F2 was the most preferred texture (8.100 ± 0.912).

The last parameter is color. The color data is not normally distributed, according to the Kolmogorov-Smirnov test ($p < 0.05$). Therefore, the Kruskal-Wallis test was performed, and the results show $p < 0.05$, indicating a significant difference between F1, F2, F3, and Brand X with a p-value of 0.005. The Mann-Whitney test results confirm that the color of F1 is significantly different from F2, F3, and Brand X. The colors of F2, F3, and Brand X do not show significant differences. Although F2, F3, and Brand X are not significantly different, F2 is the formulation most preferred by the panelists (8.200 ± 0.768).

Based on the eyeshadow evaluation test results, formulations F1, F2, and F3 satisfy the evaluation requirements for spreadability, pH, irritation, and homogeneity. The hedonic test scores showed that formulation F2 was the most preferred formulation by panelists compared to formulations F1, F3, and Brand X.

4 Conclusions

The bixin content in *Bixa orellana L.* can be extracted using ethyl acetate solvent. The extraction process used a stirred extractor at 300 rpm and 50 °C for 90 minutes, which was then evaporated at a temperature 35 °C and centrifuged to obtain the extract precipitate. The result of the bixin extracted from annatto seeds was 10.48 mg bixin/gram, while the yield was 1.2%. Compact powder eyeshadow formulations can use bixin extract, which was derived from annatto seed extraction, as a natural colorant. In this study, bixin extract was prepared at three different concentrations: 10% (F1), 20% (F2), and 30% (F3). Then, an evaluation of the eyeshadow was conducted, using Brand X as a comparison. Formulations F1, F2, and F3 satisfy the evaluation's criteria for pH, irritation, homogeneity, and spreadability, according to the eyeshadow evaluation test results. Based on the results of the

hedonic test using SPSS, the highest value for the scent parameter was obtained by the comparison product (Brand X) with an average value of 7.900. However, among the preparations, F2 showed the highest preference level with an average value of 7.150. For the texture parameter, the F2 formulation also ranked highest with an average value of 8.100. Meanwhile, for the color parameter, F2 again obtained the highest average value of 8.200. With the highest average value in the hedonic test, F2 is the most favored formulation when compared to formulations F1, F3, and Brand X.

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