MULTILEVEL CATEGORIC-ONE FACTOR OPTIMIZATION OF CREMOPHOR RH 40 AS SURFACANT IN CREAM WITH CALABASH (Crescentia cujete Linn) LEAVES ETHANOLIC EXTRACT

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ABSTRACT

Indonesia's location on the equator is exposed to a high UV index, which triggers various health problems including melanoma due to ultraviolet radiation. Melanoma is the 5th deadliest cancer that develops from the malignant transformation of melanin in oxidative stress conditions. Therefore, antioxidants are needed. Calabash is a natural exogenous antioxidant with an IC₅₀ value of 80.21 µg/mL (strong activity). Optimal formulations of cream containing calabash are determined by the concentration of ingredients and the selection of comprehensive analytical methods. This research aims to optimize cream preparations of calabash leaves ethanol extract with varying concentrations of Cremophor RH 40 as a surfactant using the multilevel categoric-one factor method. The cream was made with Cremophor RH 40 concentrations of 5%, 10%, and 15%. The cream was made by mixing the water phase (Nipagin, Glycerine, and Aquadest) and the oil phase (Cremophor RH 40, BHT, IPM, Cetostearyl alcohol, and Nipasol) after both phases reached a temperature of 60ºC in mixer speed no 1 for 15 minutes then followed by adding calabash leaves ethanolic extract until a homogeneous cream was formed. The parameters observed were organoleptic, homogeneity, the type of cream, pH, viscosity, dispersibility, and adhesion. The viscosity, dispersibility, and adhesion were chosen as the parameters for the formula optimization. The cream of calabash ethanolic extract was shown as green, homogeneous semi-solid with a soft texture and distinctive aroma, and was an oil-in-water (O/W) type cream. The average pH of 5.6 was suitable for facial skin. The viscosity value increased with increasing surfactant concentration while dispersibility decreased. The results of formula optimization obtained the optimum formula was FIII with a concentration of Cremophor RH 40 of 15% and a desirability value of 0.896.

Keywords: calabash, cream, Cremophor RH 40, optimization

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INTRODUCTION

Indonesia, as a country located on the equator with abundant exposure to sunlight, has higher health risk issues related to excessive UV exposure (Mumtazah et al., 2020). The UV index in Indonesia was, on average, 11 and reached a maximum of 13.7, which is categorized as an extreme condition with very high risk and potentially harmful to the skin (Komala, 2018; US-EPA, 2004; BMKG, 2004). A Global Burden of Disease of Solar Ultraviolet Radiation report estimates that up to 60,000 deaths a year worldwide are caused by high intensity of ultraviolet radiation (UVR) exposure (WHO, 2006). Histological proof shows that long-term UVR potentially damages DNA in keratinocytes and melanocytes, which can lead to Melanoma (Hidayati et al., 2023). Around 80% of deaths related to UVR exposure are caused by malignant melanomas (WHO, 2006). As with other cancers, melanoma is exhibited by extensive reactive oxygen species (ROS) (Zhang et al., 2022).

This is responsible for the development of melanogenesis and drug resistance and may be exacerbated by the failure of endogenous antioxidants to maintain the balance between ROS production and scavenging (Liu-Smith et al., 2014; Kaminski et al., 2022). Therefore, the body needs exogenous antioxidants to maintain homeostasis (Becker and Indra, 2023). One of the potential exogenous antioxidants is the calabash tree. Based on the research of Das et al. (2014) states that the ethanolic extract of calabash has an IC₅₀ of 80.21 µg/mL and is categorized as a potent antioxidant.

The leaves of calabash contain saponins, alkaloids, tannins, terpenes, and flavonoids. Quercetin is part of flavonoids, which function as a naturally occurring antioxidant. It is in line with research conducted by Cui et al. (2022) and Haerani et al. (2018) which shows that quercetin plays an essential role in the treatment of aging-related diseases, including melanogenesis. Quercetin of calabash leaves can prevent diseases that arise due to free radicals by binding and completing the lack of free radical electrons so that they can stabilize and inhibit chain reactions (Sofia, 2005). Aging visually appears the most on the face, so anti-aging products like creams and serums are needed. The cream is the most suitable preparation because it is easy to apply, not sticky on the face, and quickly washed with water (Sharon et al., 2013). Hedonic test on several respondents in Xenograft (2015) study stated that creams with O/W type are more preferred than creams with W/O type. Cams’ formulation and manufacturing process will affect their characteristics and physical stability. Surfactants, as the main ingredients in cream formulation, have the most significant influence on creams' physical appearance because they are vital materials that determine the formation of creams.

The optimized preparation can be obtained by optimizing the concentration of Cremophor RH 40 in the formula using the Multilevel Categoric-One Factor method in the Design Expert software trial version 22.0.6.0. The Multilevel Categoric-One Factor method in Design Expert software is used because only one factor will be varied in concentration: Cremophor RH 40 as a surfactant. Cremophor RH40, as a surfactant, can affect the cream's viscosity, dispersibility, and adhesion (Fayakun and Prihantini, 2023). Design Expert software will make it accessible for formulators to evaluate the effect of formulation variables on each preparation and more comprehensively determine the optimal formula by the degree of desirability and percentage prediction error.

METHODS

Materials and instrumentation

Calabash leaves were obtained from Gunungpati, Semarang city, Indonesia. The cream ingredients are pharmaceutical grade and consist of ethanol (96%), PEG-40 Hydrogenated Castor Oil (Cremophor® RH 40), Butylated Hydroxytoluene (BHT), Isopropyl Myristate (IPM), Cetostearyl alcohol, Glycerin, Nipagin, Nipasol, and Aquadest.

The instruments used consist of: Rotary evaporator (Heidolph), analytical balance (Ohaus), filter paper, separatory funnel, wooden stirrer, water bath, glassware equipments (Pyrex®), mixer (Maspion), pH meter (HANNA J0045115), Viscometer (Brookfield), and a set of adhesion dispersibility and homogeneity tester.

Determination of plants

The purpose of the determination is to verify the identity of the plant used in the study. Determination of calabash (Crescentia cujete Linn) was carried out at the Ecology and Biosystematics Laboratory,
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Diponegoro Semarang.

**Preparation and standardization of raw material**

The process started by harvesting dark green leaves of calabash, followed by sorting, washing, stirring, and drying at 50°C for four hours. The dried leaves of calabash were then sorted and ground to become powder. Standardization was carried out to verify the identity of dried calabash leaves powder by using visual inspection and determination of water moisture content.

**Preparation of calabash leaves ethanolic extract**

An amount 1 kg of calabash leaves powder were extracted by maceration with 7500 mL of 96% ethanol for three days. Afterwards, the macerate residues were filtered to obtain the filtrate I. Maceration was continued with re-maceration, by added the residues with 2500 mL of 96% ethanol solvent and carried out for two days. The macerate was filtered to obtain the filtrate II. Filtrate I and II were mixed and condensed using a rotary evaporator at 40°C for a thick extract (Hidayati et al., 2018a).

**Phytochemical screening test**

**Alkaloids test**

The amount of 8 mL was divided into 4 test tubes. The first tube was used as control while the other tube was added by reagent. The second tube was added by Dragendorff reagent, the third by Wagner, and the fourth by Mayer. The positive results of the Dragendorff reagent were the formation of an orange or red-brown precipitate, while the Wagner reagent was the formation of a red or brown precipitate, and the Mayer reagent was the formation of white or yellow sedimentation (Kinam et al., 2021).

**Flavonoid test**

The test was done by dividing 4 mL of test extract solution into two tubes. The first tube is the control, and the second tube is the sample test. To the second tube was added a few Magnesium powders, then warmed in the water bath for a minute, then dripped with three drops of concentrated HCl. The positive result was obtained by a brick-red color formation (Kinam et al., 2021).

**Phenolic test**

The test solution, which was as much as 4 mL, was divided into two tubes, the first tube as a control and the second tube dripped with three drops of 10% FeCl3. The positive result was shown by the solution's colouration into a green-bluish black color (Kinam et al., 2021).

**Thin layer chromatography**

Identification with the TLC used a GF254 silica plate. Each plate measures 5 x 10 cm². The first plate contains calabash leaves ethanolic extract spot as sample and rutin spot as control. At the same time, the second plate contains calabash leaves ethanolic extract spot as sample and quercetin spot as control. The first plate was eluted using acetic acid:methanol:water (15:3:2) as the mobile phase, while the second plate used methanol:chloroform (9:1) as the mobile phase.

**Preparation of calabash (Crescentia Cujete Linn) leaves ethanolic extract cream**

Calabash leaves ethanolic extract cream formula is presented in Table I. Preparation of the oil phase was started by weighing Cremophor RH40, Cetostearyl alcohol, Nipasol, BHT, IPM, then put into a porcelain cup and heated on a water bath while stirring until homogeneous. Meanwhile, the preparation of the water phase began by weighing nipagin, glycerine, and distilled water, which were subsequently heated in a water bath. After both phases reached a temperature of 60°C, the oil phase was dropped into the water phase while stirring using a mixer at a speed of 1 for 15 minutes until the cream basis was formed. The last preparation stage was adding calabash leaves ethanolic extract into the cream until a homogeneous cream was formed.
Table I. Formula of Calabash Leaves Ethanolic Extract Cream

<table>
<thead>
<tr>
<th>Materials</th>
<th>Concentration (%)</th>
<th>FI</th>
<th>FII</th>
<th>FIII</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calabash Leaves Ethanolic Extract</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>Active substance</td>
</tr>
<tr>
<td>BHT</td>
<td>0,05</td>
<td>0,05</td>
<td>0,05</td>
<td></td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Cremophor RH40</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td>Surfactant</td>
</tr>
<tr>
<td>Cetostearyl Alcohol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td>Thickener</td>
</tr>
<tr>
<td>Glycerine</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
<td>Emollient</td>
</tr>
<tr>
<td>Nipagin</td>
<td>0,02</td>
<td>0,02</td>
<td>0,02</td>
<td></td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Nipasol</td>
<td>0,18</td>
<td>0,18</td>
<td>0,18</td>
<td></td>
<td>preservative</td>
</tr>
<tr>
<td>IPM</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td>Oil phase</td>
</tr>
<tr>
<td>Aquadest</td>
<td>add to 100</td>
<td>add to 100</td>
<td>add to 100</td>
<td>Water phase</td>
<td></td>
</tr>
</tbody>
</table>

Physical Characteristics of Calabash Leaves Ethanolic Extract Cream

Organoleptic and homogeneity

An organoleptic test was applied to all formulas by observing changes in shape, color, and odor of the calabash leaves ethanolic extract cream preparation. The homogeneity test was carried out by applying 1 gram of cream to a piece of transparent glass and observing. A homogeneous preparation was defined by a smooth appearance without coarse particles and phase separation (Ansel, 1989; Ditjen POM, 1985).

Type of cream

The method used to observe the cream type is the dilution method, which involves dissolving the cream in water. If the cream is dissolved continuously in water, it is an O/W cream. In contrast, if it was separated into two phases, it is a W/O cream (Voigt, 1984).

pH

The pH test was conducted using a pH meter at room temperature. Before use, the electrodes were washed and rinsed with distilled water and dried. The device was calibrated using standard aqueous solutions of pH 4 and pH 7 (Depkes RI, 2020).

Dispersibility

The calabash leaves ethanolic extract cream was weighed at 0.5 g, placed in a glass plate, and then given a weight of 50, 100, 150, 200, 250, and 300 grams. It was left for 60 seconds for each change in weight. The diameters of several edges spreading were calculated.

Adhesion

The adhesion test was carried out by weighing the preparation as much as 0.5 grams and placing it on a glass object with covered glass placed above. The glass object was then mounted on the test device and given a 1 kg load for 5 minutes, and then released with a load weighing 80 grams. The time was recorded until the two glass objects were released (Allen, 1998).

Viscosity

The viscosity test was carried out with a Brookfield Viscosimeter, with the spindle dipped into the cream. Viscosity was defined by observing the viscosity number on the screen, which pointed to a constant number (Lachman et al., 1994).

Formula optimization

The design of the calabash leaves ethanolic extract cream preparation formula was carried out using Design Expert software trial version 22.0.6.0 with the multi-level categoric-one factor factorial design method. Cremophor RH 40 factor consists of low level (5%), medium level (10%), and highest concentration (15%). The results of the viscosity, adhesion, and dispersibility test were optimized as these parameters determined the characteristics of the cream.
RESULTS AND DISCUSSION

Determination of plant

From the determination results, it was known that the sample used in this study is *Crescentia cujete* Linn with Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Asteridae, Order: Shropulariales, Family: Bignoniaceae, Genus: *Crescentia*, Species: *Crescentia cujete* L. The regional name is Calabash with Determination Key: 1b-2b-3b-4b-6b-7b-9b-10b- (Goal 8. Scattered single leaf plants) -109b-119b-120b-128b-129b-135b-136b-139b-140b-142b-143b-146b-154b-155b-156b-162b-163b-167b-169b-171b-177b-179b-187b-189b-190b-191b-192b-193a-194b-(Family 113. Bignoniaceae)-1b-3a-(Genus *Crescentia*)-(Crescentia cujete*L.)*.

Calabash leaves ethanolic extract

The moisture content of a dried calabash leaves powder was 7.8%. The acceptance limit of moisture content is under 10% (Depkes RI, 2008). If the moisture content is more than 10%, it will cause enzymatic processes and potentially increase microbial and fungal growth that can damage the quality of the extract (Paris and Moyse, 1976). The yield of calabash leaves ethanolic extract was 9.71%. It was higher than similar research conducted by Hidayati et al. (2018b), which obtained an extract yield of 4.75%. Organoleptic results of calabash leaves ethanolic extract obtained a concentrated green color, thick shape, the distinctive odor of calabash and bitter taste. In the test of water-soluble and ethanol-soluble content, it was found that the ethanol-soluble content was higher than the water-soluble content (53.33% > 16.67%).

Phytochemical screening

Based on the results of phytochemical tests carried out on calabash leaves that are shown in Table II. Based on the results, calabash leaves had secondary metabolite compounds, such as alkaloids, phenolics, and flavonoids. It is in line with research conducted by Kinam (2021) that extracts and fractions of calabash leaves contain alkaloids, flavonoids, phenol, saponin, and steroid or triterpenoid compounds.

<table>
<thead>
<tr>
<th>Secondary metabolite grup</th>
<th>Reagent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Concentrated HCl + Mg powder + amyl alcohol</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>FeCl₃</td>
<td>+</td>
</tr>
</tbody>
</table>

Description: (+) = Positive contain metabolite compound

Thin layer chromatography

The test on the flavonoid compound class of calabash leaves was compared with two standards: rutin and quercetin. The TLC results are shown in Figure 1. Using the citro borate reagent, the chromatogram results produced a yellow-green colour, indicating the presence of flavonoid compounds in the ethanol extract of calabash leaves. This was in line with research by Bupu (2022), which stated that compounds containing quercetin show yellowish-green fluorescence under UV light at 366 nm. The Rf values obtained were 0.9375 for the target compound and 0.8375 for the quercetin comparator. The Rf value of the target compound differed from that of the rutin comparator, which was 0.5. Thus, it can be concluded that the flavonoid compound is quercetin, as the Rf value of the ethanolic extract of calabash leaves (0.8375) was close to that of the quercetin comparator (0.9375).
Description: Plate R = Rutin; Plate Q = Quercetin; C = Control. S = Sample

Figure 1. Thin layer chromatography test results at (a) with spotting agent (b) UV light 254 nm (c) UV light 366 nm.

**Organoleptic and homogeneity**

The organoleptic test results of the cream made from calabash leaves ethanolic extract showed consistent shapes, colors, and odors, with a heavy cream consistency, green colour, and a typical Calabash leaf smell. This is in line with research conducted by Nurjanah et al., (2021), which found that the cream has a distinctive extract odor and that the cream's color matches the color of the calabash leaf extract. Organoleptic test results are shown in Figure 2.

![Organoleptic Test Results](image)

**Figure 2. Organoleptic test results of cream Calabash Leaves Ethanolic Extract (a) FI, (b) FII, (c) FIII.**

Visually, the increase in Cremophor RH 40 concentration did not affect the organoleptic properties and homogeneity. The homogeneity test results for the cream (FI, FII, and FIII) indicated a homogeneous mixture without coarse particles or phase separation. This aligns with research by Arisanty and Anita (2018) which stated that a cream preparation must be homogeneous to prevent skin irritation and ensure even distribution. This is in line with research conducted by Nurjanah et al. (2021) that the cream shows good or homogeneous results because there are no visible coarse grains. The homogeneity test results are shown in Figure 3.

**Type Cream**

The cream type determination test results for the ethanolic extract of calabash leaves in FI, FII, and FIII showed that the cream can be dispersed evenly in distilled water, proving that it is an O/W (oil-in-water) type cream. These results are consistent with the research by Kusuma et al. (2022), which states that O/W type cream will mix homogeneously with distilled water and is easier to wash off because the outer phase is water.
The pH test results of the cream made from calabash leaves ethanolic extract in FI, FII, and FIII showed an average pH of 5.6. This value is within the facial skin pH range of 4.5-6.5, making the cream suitable for use without causing irritation or scaly skin (Tranggono and Latifah, 2007). Increasing the concentration of Cremophor RH 40 does not affect the pH of the cream preparation, as the pH of Cremophor remains stable at concentrations above 3% in water, around pH 5.5-5.7 (Ataman-Chemicals, 2020). pH test results can be viewed in Figure 4.

**Dispersibility**

The dispersibility test conducted on the cream made from calabash leaves ethanolic extract in FI, FII, and FIII showed that an increasing concentration of Cremophor RH 40 decreases dispersibility, which is inversely proportional to viscosity. This is consistent with research by Mudhana and Pujiastuti (2021), which states that higher surfactant concentrations lower dispersibility. Additionally, research by Fayakun and Prihantini (2023) showed that increasing Cremophor RH 40 concentration from 10% to 30% resulted in higher viscosity and lower dispersibility. The results of the dispersibility test are shown in Figure 5.

**Adhesion**

The results of the adhesion test on FI, FII, and FIII are shown in Figure 6. Figure 6 indicates that FI, FII, and FIII have a short adhesion time, suggesting that the active compound provides a fast onset time for effectiveness. Creams with shorter adhesion times are more acceptable for use on facial skin due to increased comfort (Elcistia and Zulkarnain, 2019).

**Viscosity**

The viscosity test results of the cream made from calabash leaves ethanolic extract in FI, FII, and FIII are shown in Figure 7. The higher the concentration of Cremophor RH 40, the greater the viscosity. FIII has a higher viscosity compared to FII, and FII has a higher viscosity compared to FI.
This aligns with research by Fayakun and Prihantini (2023), which showed that increasing Cremophor RH 40 concentration increases the viscosity of the AG-KS molecular complex nanoemulsion.

![Figure 5. Dispersibility value of cream of calabash leaves ethanolic extract](image1)

![Figure 6. Adhesion value of cream of calabash leaves ethanolic extract](image2)

![Figure 7. Viscosity value of cream of calabash leaves ethanolic extract](image3)
Formula optimization

The response analysis of the adhesion parameter showed that the data was normally distributed, indicated by the straight triangle on the red line. However, the data was not homogeneous, characterized by 1 point away from the red line. Then, in the ANOVA test, the p-value shows 0.1464 > 0.05, which means the data is not significant; with these results, it is concluded that the adhesion is not influenced by the variation of Cremophor RH 40. The adhesion of the two treatments compared none showed significant differences because the prob>|t| value was more than 0.1.

The response analysis of the dispersibility parameter shows that the data is normally distributed, as indicated by the straight triangle on the red line. In addition, the data is homogeneous and characterized by the distribution of points near the red line. Then, in the ANOVA test, the p-value shows 0.0438 < 0.05, which means the data is significant; with these results, it is concluded that the adhesiveness is influenced by variations in Cremophor RH 40. The adhesiveness of the two treatments compared in FI vs FIII has a significant difference because the prob>|t| value is less than 0.05. Meanwhile, FI vs FII and FII vs FIII do not have significant differences because the prob>|t| value is more than 0.1.

The viscosity parameter response analysis shows that the data is normally distributed, as indicated by the straight triangle on the red line. However, the data is not homogeneous, marked by 1 point away from the red line. Then, in the ANOVA test, the p-value shows 0.2828 > 0.05, which means the data is not significant; with these results, it is concluded that viscosity is not influenced by variations in Cremophor RH 40. The viscosity of each of the two treatments was compared, none of which showed significant differences because the prob>|t| value was more than 0.1.

Figure 8. Formula optimization test results of cream calabash leaves ethanolic extract

Furthermore, to achieve the most optimal formula that has minimal adhesion, minimal dispersibility, and maximum viscosity, formula optimization is out, as shown in Figure 8. The results show that the optimum formula obtained is formula 3 with 15% Cremophor RH 40 concentration, which has an average adhesion of 1,000 seconds, an average dispersibility of 6,200 cm, and an average viscosity of 9094.667 cP. Based on the 15% variation of Cremophor RH 40, it shows the highest desirability number of 0.896. In the optimization analysis, a desirability value close to 1 indicates the most optimum condition (Ayuningtyas et al, 2022). The desirability value achieves the desired optimum value of 0.896, which means that it enters the range of desirability values, which is 0-1 (Saryanti et al., 2019). This research is in line with the research of Fayakun and Prihantini (2023), that the formula with 15%-20% cremophor concentration has an optimum desirability value because it enters the range and is close to 1. When compared, FIII is the most optimum 0.896 because it is closer to 1 than FII, which has a desirability value of 0.615, and FI has a desirability value of 0.331.

CONCLUSIONS

The cream of calabash ethanolic extract is green, homogeneous, semi-solid with a soft texture, has a distinctive aroma and is an oil-in-water (O/W) type of cream. The average pH was 5.6, which is suitable for facial skin. The viscosity value increased by increasing surfactant concentration while dispersibility decreased. The formula optimization using the multilevel categoric-one factor factorial
Multilevel One-Categoric Factor... Riyanti et al., 2024

design method resulted in an optimal desirability value of 0.896, with the optimal concentration of Cremophor being 15%.

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