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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Development and Microbiological Evaluation of Natural Diaper Rash (Diaper Dermatitis) Cream Formulations

Doğal Bebek Bezi Pişiği (Bebek Bezi Dermatidi) Krem Formülasyonlarının Geliştirilmesi ve Mikrobiyolojik Değerlendirilmesi

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Abstract

Backround: Rosa damascena Mill, released into the environment as waste in rose products production facilities, contains antioxidant, antimicrobial, and antiseptic phenolic components. It is the development of an effective natural cream formulation for baby diaper rash by taking advantage of the antimicrobial properties of rose pulp and adding natural ingredients (zinc oxide ZnO, natural oils, and beeswax).

Material and Methods: The emulsification method was used to prepare diaper rash cream formulations. Rotational type viscosity determination was performed to examine the rheological behavior of the formulations. In selecting the optimum formulation, pH, viscosity, hydrophilic-lipophilic balance, and physical appearance of the product were considered. To examine its stability properties, its stability was examined in three different environments in line with ICH directives for 6months. The optimum DR-C-7 formulation was subjected to physicochemical and stability tests.

Results: It was observed that the DR-C-7 formulation had a viscosity between 9,820 and 26,130 (Pa.s) in terms of rheological properties. As a result of the challenge test, no microbiological units were found. At the end of a 6-month stability study under different conditions, it was observed that it retained all its features.

Conclusion: It was concluded that R. damascena pulp, which has important phenolic contents such as phenylethyl alcohol, flavonoids, and terpenoids, can be used for thick products such as diaper rash cream with its antioxidant antimicrobial properties.

Keywords: Dermocosmetics, Rose Damescana Pulp, Baby Rash, Emulsion, Microbiological Analysis

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Öz

Amaç: Gül ürünleri üretim tesislerinde atık olarak çevreye salınan Rosa damascena Mill, antioksidan, antimikrobiyal ve antiseptik fenolik bileşenler içermektedir. Gül posasının antimikrobiyal özelliklerinden yararlanılarak ve doğal bileşenler (çinko oksit ZnO, doğal yağlar ve balmumu) eklenerek bebek pişikleri için etkili bir doğal krem formülasyonunun geliştirilmesidir.

Gereç ve Yöntem: Pişik kremi formülasyonlarını hazırlamak için emülsifikasyon yöntemi kullanılmıştır. Formülasyonların reolojik davranışını incelemek için rotasyonel tip viskozite tayini yapılmıştır. Optimum formülasyonun seçiminde pH, viskozite, hidrofilik-lipofilik denge ve ürünün fiziksel görünümü dikkate alınmıştır. Stabilite özelliklerini incelemek için, ICH direktifleri doğrultusunda üç farklı ortamda 6 ay boyunca stabilitesi incelenmiştir. Optimum DR-C-7 formülasyonu fizikokimyasal ve stabilite testlerine tabi tutulmuştur.

Bulgular: Reolojik özellikler açısından DR-C-7 formülasyonunun 9,820 ile 26,130 (Pa.s) arasında bir viskoziteye sahip olduğu gözlenmiştir. Zorlama testi sonucunda herhangi bir mikrobiyolojik birime rastlanmamıştır. Farklı koşullar altında yapılan 6 aylık stabilite çalışması sonunda tüm özelliklerini koruduğu gözlenmiştir.

Sonuç: Feniletil alkol, flavonoidler ve terpenoidler gibi önemli fenolik içeriklere sahip olan R. damascena pulpunun antioksidan antimikrobiyal özellikleri ile pişik kremi gibi kalın ürünlerde kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Dermokozmetik, Rose Damescana Pulpası, Pişik, Emülsiyon, Mikrobiyolojik Analiz

INTRODUCTION

Diaper dermatitis, commonly referred to as diaper rash, is a skin problem that occurs as a result of closure, moisture and irritation in and around the perineum, sub-perineum (1, 2) It is the most common skin disease with a rate of 7-35% in infants and usually develops in the 9-12th months (1). In adults, the skin acts as a protective barrier against all kinds of external factors. However, proper and effective protection in infants usually occurs after 1 year of age. Compared to adult skin, infant skin is thinner, less pigmented, less tolerant to heat and less thermoregulated. Prevention of nappy rash involves strengthening the skin barrier and eliminating the factors that cause inflammation. In this context, diaper rash cream was developed using the antioxidant, antimicrobial and antiseptic phenolic components of Rosa damascena Mill pulp, which is left to the environment as waste in rose product production facilities (3, 4). Microorganisms alone are not effective in the development of nappy dermatitis. However, the interaction of other factors facilitates the access of microorganisms to the epidermis through the damaged stratum corneum (SC) layer, which increases the risk of secondary infections caused by fungi and bacteria (5). When secondary infection develops, the course of gland dermatitis becomes more severe (6). In addition, the use of broad-spectrum antibiotics for various reasons increases the risk of developing gland dermatitis (2). Microbiological

contamination of cosmetic products is important in terms of both posing a risk to consumer health and causing economic loss due to changes that may occur in the product (odour and gas formation, colour and viscosity changes, etc.) (7). Studies show that cosmetic products are mostly exposed to contamination during use by the consumer. Using the products after the expiry date specified on the label, use by more than one person, wetting with saliva, inserting a finger or contaminated object, being in contact with air are among the most important reasons for contamination during use. Microorganisms that frequently cause contamination in cosmetic products are reported as P. aeroginosa, S. aureus, Enterobacter sp., E. coli, K. pneumonia, S. epidermidis, C. albicans, Aspergillus sp. (8). The presence of these microorganisms poses a danger to the health of users. Therefore, in order to prevent microbial growth, some substances with different chemical structures are added as protection against contamination (9). Today, preservative efficacy tests are carried out according to different methods determined by organisations such as the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP) (10). Microbiological evaluations of these products offered to the market are mandatory for the health of the target audience. There are many methods (pouring, smearing and dripping) for microbiological analysis (11). The aim of this study was to develop an effective natural cream formulation for baby rash by utilising the antimicrobial properties of rose pomace and adding natural ingredients (zinc oxide ZnO, natural oils and beeswax).

MATERIALS AND METHODS

Rose Pomace (Local Producer, Türkiye), Zinc Oxide (Galenik, Türkiye), White Beeswax (Galenik, Türkiye), Sorbitan monooleate (Galenik, Türkiye), Olive Oil (Talya, Türkiye), Lanolin (Doğa Pharm., Türkiye), Polysorbate 80 (Galenik, Türkiye), Devices used: Milwaukee MW150 max (Szeged-Hungary), Rotational Viscometer PCE-RVI 10(Meschede-Germany), Elektromag M5040 PS (Çerkezköy – Türkiye), Mechanic Stirrer (Isolab, Germany), Heated magnetic stirrer (Isolab, Germany).

Method

The main component of the formulation is *Rosa damascena* Mill pulp. It was prepared as a water/oil type emulsion system by adding water phase to the oil phase. The oil phase ingredients (olive oil, almond oil, lanolin, beeswax, calendula oil, emulsifiers) were weighed from low to high on an analytical balance and melted at 70°C until completely liquefied. Water phase materials (magnesium sulphate, EDTA, glycerin, water) were weighed, heated to 70°C and slowly poured over the oil phase. The mixture was homogenised in a high speed homogeniser for 5 minutes. Zinc oxide and vitamin E were then added to the mixture and homogenised at 2000 rpm for 5 min (12). The formulation components are given in Table 1. Eight different formulations were prepared by changing the systems in the diaper rash cream. The distribution of the formulations was checked for colour, rheological properties and other physicochemical properties. Microbiological analysis of the resulting formulation was performed using the pour plate method. Total bacterial and fungal analyses for *Staphylococcus aureus*,

aerobic mesophilic (bacteria, yeast moulds), *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* were reported as CFU/g.

Formulation study

Cream formulations are classical emulsion formulations. Emulsion components include oil phase, water phase and emulsifiers. While developing rose pulp nappy rash creams, it was aimed to prepare suitable formulations in which the rose pulp could be completely emulsified in the cream. The oil phase components were selected in different concentrations and the optimum concentration ratio was tried to be determined for the water phase where the active components of the emulsion are present. Antioxidant, chelating agent and preservative concentrations were kept constant during formulation design. Olive oil and almond oil were used in three different concentrations, zinc oxide, beeswax and lanolin were used in two different concentrations. In addition, the effectiveness of emulsifiers was evaluated by using concentrations in accordance with and opposite to the HLB calculation. In total, eight different formulations were developed and physicochemical studies were carried out to select the optimum formulation (Table 1).

Table 1. Composition of eight different diaper rash cream formulations containing *Rosa Damescana*

Ingredient	DR-C-1	DR-C-2	DR-C-3	DR-C-4	DR-C-5	DR-C-6	DR-C-7	DR-C-8
Olea Europa oil	10.00	15.00	20.00	10.00	15.00	20.00	15.00	15.00
Almond Oil	10.00	10.00	10.00	20.00	20.00	20.00	15.00	15.00
Beeswax	4.00	4.00	4.00	5.00	5.00	5.00	5.00	5.00
Lanolin	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00
Sorbitan monooleate	3.50	4.24	4.99	5.23	6.07	7.20	5.23	2.17
Calendula officinalis extract	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zinc oxide	10.00	10.00	10.00	10.00	10.00	5.00	5.00	5.00
Rose pulp	2.00	2.00	2.00	5.00	5.00	5.00	5.00	5.00
Polysorbate 80	1.50	1.76	2.01	1.97	2.33	2.20	2.17	5.23
Magnesium sulphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BHT	0.05	0.05	0.05	0.05	0.05	0.05	1.00	1.00
EDTA	0.05	0.05	0.05	0.05	0.05	0.05	1.00	1.00
Ethylhexylglycerin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glycerin	5.00	5.00	5.00	5.00	5.00	10.00	10.00	10.00
D.Water	46.90	40.90	34.90	32.70	26.50	20.50	30.60	30.60

Microbiological analyses

The formulation developed for microbiological analysis within the scope of the study was disinfected with 70% ethanol. To dissolve the product, 5 g/L polysorbate 80 was added to 90 ml TSP (Buffered Sodium Chloride Peptone), 10 g of sample was added and left to dissolve in a water bath for 10-15 minutes. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}) were prepared by transferring 1 ml of the sample suspension to 9 ml of TSP using the pour plate method. The dilutions were repeated twice by inoculating 1 ml of the diluted tubes into a 90 mm petri dish. Then 15-17 ml of agar medium cooled to 45°C in a water bath was poured into the petri dishes and left to freeze. Tyriptic Soy Agar (TSA) was used for the total number of aerobic mesophilic microorganisms which were left at 30-35°C for 3-5 days. SDA medium was

used for total yeast and mould counts and the media were incubated at 20-25°C for 5-7 days. In case of growth, the calculation formula is used to count colonies visible to the naked eye. This formula is as follows:

CFU/ml = Total number of colonies obtained x dilution factor/ Sample volüme

Enrichment

Ten g of the sample dissolved in buffered sodium chloride peptone was transferred to 90 ml of Tyriptic Soy Broth (TSB). This medium contains lecithin and polysorbate required for neutralisation and is a general producer medium. After thorough shaking, the medium was incubated at 30-35°C for 18-24 hours (maximum 72 hours). After incubation, a selective medium was used. Enrichment was performed for *E. coli, P. aeruginosa* and S*. aureus. For C. albicans,* 10 ml (1 g or ml) of the sample dissolved in TSP was transferred to 90 ml Sabouraud Dextrose Broth (SDB). After shaking well, it was incubated at 30-35°C for 72 hours (maximum five days). After incubation, a selective medium was used.

Investigation of Aerobic Mesophilic Bacteria

After enrichment, 1 ml of TSB medium was taken and placed in sterile petri dishes. 5 ml of medium was added to Tryptone Glucose Extract Agar (TGEA) medium cooled to 45°C, mixed and inoculated with the sample in duplicate and allowed to solidify. After solidification, it was incubated at 37°C for 48 hours. In case of growth at the end of incubation, the number of colonies formed is calculated taking into account the dilution factor.

Investigation of The Presence of Escherichia Coli

After enrichment, 1 ml of TSB medium was taken and placed in sterile petri dishes. Then 5 ml of medium was added to Macconkey Agar (MCA) medium, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation for The Presence of Staphylococcus Aureus

After enrichment, 1 ml of TSB medium was taken and placed in sterile petri dishes. 5 ml of medium was added to Mannitol Salt Agar (MSA) medium cooled to 45°C, inoculated with the sample in duplicate and allowed to solidify. Incubated at 30-35°C for 24 hours (maximum 48 hours).

Investigation of The Presence of Pseudomonas Aeruginosa

After enrichment, 1 ml of TSB medium was taken and placed in sterile petri dishes. 5 ml of medium was added to Cetrimide Agar (CA) medium cooled to 45°C, mixed and the medium was inoculated with the sample in duplicate and allowed to solidify. Plates were incubated at 25˚C for 5 to 7 days.

Rheological Studies

Apparent viscosity was determined using a PCE-RVI-10 rotational viscometer (PCE Instruments, Hamburg, Germany). Rotational rheometer measures viscosity by calculating the torque required to rotate the spindles immersed in the fluid. The applied torque is related to the viscous friction on the shaft and therefore the viscosity of the fluid. Samples were measured with an L2 spindle at 6 rpm for 120 sec. was carried out throughout. The slip rate is calculated taking into account the dimensions of the shaft,

the rotation speed and the gap between the shaft and the container (formulation 1-2). The gap between the shaft and the container was set to 1.25 mm, resulting in a ratio of 1.2 between the container diameter and the shaft(13). The sliding speed was calculated according to the formulation 1-2. Whether the difference between the values of the formulae was significant or not was evaluated using One-Way Anova test.

$$y = 2 \times \frac{2 \times \pi \times Ni}{60} \times \frac{R_0^2}{R_0^2 - Ri^2} \quad (1)$$

In the equation, "y" is the sliding speed in s-1, "Ni" is the rotation speed in rpm, and "R0" and "Ri" are the radius of the cup and shaft in mm, respectively. Apparent viscosity values were plotted as a function of shear rate and fitted to Eq x according to the Ostwald-de Waele relationship (14).

$$\eta = K.\gamma^{n-1} \quad (2)$$

Here, K is the viscosity coefficient n is the flow behavior index calculated from exponential regression (14).

Calculation of The İnterval of Safety (Mos)

The safety margin value (MOS) of the diaper rash cream formulation is determined in the Turkish Medicines and Medical Devices Agency (TITCK) Cosmetic Guide 3, as specified in the skin surface area (860 cm2), application frequency (1/day) and daily exposure level (according to the product type). It was taken as 2.16 g/day). Systemic exposure dose (SED) was calculated with formula 3 and safety margin values were calculated with formula 4. When calculating dermal absorption, calculations were made assuming that 100% of the product would be absorbed as the worst exposure scenario for all raw materials. MoS value is expected to be higher than 240 in baby products. In this study, these criteria were taken into consideration since the formulation of baby nappy rash cream was carried out.

$$SED = \frac{DAa\ (\mu g/cm2) \times 10\text{-}3mg/\ \mu g \times SSA\ (cm2) \times F(gün\text{-}1)}{60} \quad (3)$$

$$MoS = \frac{PODsys}{SED*\%kons.} \geq 100 \text{ (yetişkinler için)} \quad (4)$$

Stability Study

Stability studies were checked for appearance, color, pH, viscosity changes and microbiological growth in the refrigerator, room and oven (45 ^{0}C) for 6 months, in accordance with ICH ant TITCK directives (18).

RESULTS

Baby barrier cream formulations were tested in terms of physical-chemical, stability and harmful microorganisms on the skin of infants and it was concluded that they comply with microbiological limit values. As a result of the study, characterisation and safety assessment were completed and it was decided that DR-C-7 formulation was the most suitable formulation among eight formulations in terms of appearance, flow properties and stability.

Microorganism Analysis Result

No growth was detected in our tested shampoo product. No growth was observed in this product on the 14_{th} and 28_{th} days following the effect of the preservative

(Table 2).

Table 2. Microbiological test results of cosmetic sample

Microorganisms	Conclusion
Total number of aerobic mesophilic microorganisms (Bacteria, mold, yeast)	≤1 x 102 cfu/ g or ml
Yeast and mold	≤10^2 cfu
Escherichia coli	1 g ve ml not found
Pseudomonas aeruginosa	1 g ve ml not found
Staphylococcus aureus	1 g ve ml not found
Candida albicans	1 g ve ml not found

Physicochemical test results

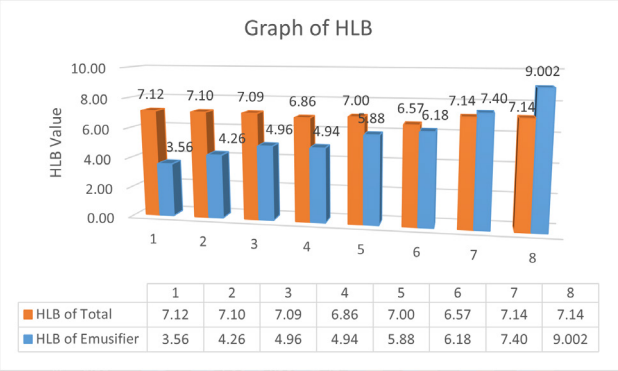
The following table presents the HLB calculation results and measurements of viscosity, yield stress, and flow rate, which are rheological properties, for formulations suitable for infant skin with a skin pH range of 6.2-7.5. Whether the difference between the values of the formulae was significant or not was evaluated using One-Way Anova test. According to the results of statistical analysis, it was found that there was no significant difference between the formulations in terms of pH values ($p>0.05$). HLB values and viscosity values of the formulations were statistically significant ($p<0.05$).

Table 3. Appearance, pH, HLB and rheological results of the formulations (n=3).

Formulation code	Appearance	pH Measurment Result	Oil Phase HLB	Emulsifier HLB	Viscosity (Pa.s)	Shear Rate (1/sec)	Shear Stress (D/cm²)
DR-C-1	Homogenous Cream	7.201±0.10	7.52	3.76	31.23±5.67	0.14	4.37±1.12
DR-C-2	Homogenous Cream	7.243± 0.12	7.43	4.46	34.54±5.22	0.11	4.83±1.03
DR-C-3	Homogenous Cream	7.320±0.14	7.37	5.16	35.45±4.18	0.12	4.96±0.82
DR-C-4	Homogenous Cream	7.137 ± 0.09	7.22	5.2	30.62±5.78	0.12	6.38±1.14
DR-C-5	Homogenous Cream	7.212 ± 0.08	7.26	6.1	37.51±5.22	0.11	6.09±1.03
DR-C-6	Homogenous Cream	6.949 ± 0.11	6.81	6.4	48.20±6.21	0.14	6.74±1.22
DR-C-7	Homogenous Cream	6.992 ± 0.13	7.43	5.5	38.2±4.37	0.12	5.34±0.86
DR-C-8	Homogenous Cream	-	7.43	8.78	-	-	-

HLB calculation results

The calculation of the amount of emulsifier to be used in cream formulations was carried out based on the HLB values of the lipophilic and hydrophilic emulsifiers used. In order to verify the HLB calculation of DR-C-7 and DR-C-8 creams with the same ratio of oil phase and water phase in the formulations, a reverse amount of emulsifier experiment was performed. As a result, it was observed that the phases were not dispersed in each other and phase separation was observed.



Graph 1. HLB calculation graph for all cream formulations.

According to the above results, it is seen that the emulsifier used in the 8th formulation was incorrectly selected. Considering the HLB values in the other 7 formulations, it

was determined that the emulsifier selection was correct and no phase separation was observed.

Rheological Studies Result

The DR-C-7 optimum diaper rash cream formulation resulted in n<1, showing shear thickening according to the Ostwald-de Waele and exhibited Pseudo-Plastic flow. According to the results, the shear rate is between 0.12 s^{-1} and 24.64 s^{-1}, the shear stress is between 271.01 and 2419.35(D/cm^2), and the viscosity values are 220 and 9.82 Pa.S.

Table 4. Viscosity, shear stress, shear rate results of the optimum formulation DR-C-7

Viscostiy (Pa.s)	Shear Rate (1/sec)	Share Stress (D/cm^2)
220±14.64	0.12	0.27±0.02
120±10.50	0.25	0.30±0.03
65.2±8.95	0.62	0.40±0.06
45.3±4.76	1.23	0.56±0.06
38.2±4.37	2.46	0.94±0.11
25.95±1.06	4.93	1.28±0.05
18.04±9.11	12.32	2.22±1.12
9.82±4.50	24.64	2.42±1.11

Graph 2. Graph of viscosity and shear stress versus shear rate of the optimum formulation GP-C-7 (n=3)

Safety Interval Results

The safety assessment results of the DR-C-7 formulation were calculated according to fromulation 3 and 4 and are given below in the table. According to the cosmetic legislation, the MoS (safety limit) value should be equivalent to or above 240 in the evaluations made with the ratios of the concentrations of the ingredients in it, since it is foreseen to be used in infants. Olive oil, Almond Oil, Rose pulp, Shea Butter, Calendula Oil, Calendula Oil, Beeswax do not have NO(A)EL values since the raw materials are natural and used as food. In this case, they are considered safe for topical applications regardless of their concentration of use.

Table 5. MoS values of the components of DR-C-7 fomulation

İngredient	POD_{sys}	MoS
Lanolin	5000	3472>240
Sorbitan monooleate	2600	502>100
Zinc oxide	7950	1104>100
Polysorbate 80	5000	1328>240
Magnesium sulphate	1029	1429>240
Antioxidant (BHT)	2000	5555>240
EDTA	800	1111>240
Ethylhexylglycerin	2000	13889>240
Glycerin	12600	1750>240

Stability Results

Stability studies were carried out in refrigerator, room and oven (45 ^{0}C) for 6 months in accordance with ICH directives and TİTCK cosmetic regulation. The appearance, colour, pH, viscosity changes and microbiological growth were checked. Rose pulp nappy rash cream maintained its physicochemical properties in all environments. After organoleptic controls, it was observed that the specific appearance and colour remained the same from day 0 to 6 months. It was observed that the pH value was in the range of pH 5.5-5.7 under all conditions in accordance with the pH value of the skin, which did not leave any burning sensation for the skin. For the control of microbiological growth, the protective efficacy test (45^{0}C) was carried out in an oven and no growth was found.

Table 6. Stability results; Appearance, color, odor, and microbiological growth results as of Day 0, Month 3, and Month 6

Stability Condition	Control Period	View	Colour	Viscostiy(Pa.S)	Mıcrobıologıcal Growth (45°C) In Incubator
Room Conditions	0 .Month	Homogenıc Cream	Specıfic	25,95±1,06	No Reproductıon
	3 .Month	Homogenıc Cream	Specıfic	25,55±1,31	No Reproductıon
	6 .Month	Homogenıc Cream	Specıfic	25,34±1,36	No Reproductıon
Incubator (45°C)	0 .Month	Homogenıc Cream	Specıfic	22,05±2,10	No Reproductıon
	3 .Month	Homogenıc Cream	Specıfic	21,55±1,12	No Reproductıon
	6 .Month	Homogenıc Cream	Specıfic	20,95±2,16	No Reproductıon
Refrigerator	0 .Month	Homogenıc Cream	Specıfic	26,25±0,96	No Reproductıon
	3 .Month	Homogenıc Cream	Specıfic	26,42±0,78	No Reproductıon
	6 .Month	Homogenıc Cream	Specıfic	26,75±0,96	No Reproductıon

DISCUSSION

Diaper rash is the most common skin problem in childhood, which can be seen in all nappied infants (15). In the literature, it is reported that the incidence is affected by many factors and varies between 7% and 35% (6). The cause of diaper rash is irritation of the skin as a result of excessive moisture and friction, and the pH of the skin changes from acidic to alkaline and becomes colonised with Candida albicans and bacteria (1). The cosmetic manufacturer must ensure that the devices and materials to be used in production are clean and the products are free from pathogenic microorganisms in accordance with Good Manufacturing Practices (GMP) and Microbiological Quality Management. In addition, procedures should include microbiological control of raw materials, bulk and finished products, materials used in packaging, personnel, equipment, preparation and storage rooms (16). The reason for diaper rash is irritation of the skin as a result of excessive moisture and friction. As a result of not changing the diaper frequently, the in contact with urine changes from acidic to alkaline and becomes colonized with microorganisms. Alkaline pH damages the stratum corneum layer by activating the protease and lipase enzymes in the stool (17). In preventing diaper rash; Changing the baby's diaper frequently, ventilating the diaper, choosing diapers with high absorbency capacity, not tying the diaper tightly, not using alcohol-containing cleansing wipes, instead cleaning the area with warm water after each defecation, applying a thin layer of protective creams at each diaper change and avoiding damaging the respiratory tract. It is of great importance to avoid the use of powder because it can cause damage (18). In our study, when the content of diaper rash protective creams was examined; Zinc oxide, lanolin were most commonly used for this purpose (20). In our study, opening the sample, taking the appropriate amount and transferring it to TSP was performed under aseptic conditions in a biosafety cabinet. This type of cream forms a lipid layer on the skin and protects the baby's skin from harmful microorganisms and irritants. Zinc oxide, which has low toxicity, kills microbes on the skin surface with its antiseptic properties, improves the general appearance of the skin and helps to reduce irritation caused by allergic reactions (21). In our study, zinc oxide was used in 8 different formulations,

researches were carried out on its physical and chemical properties, diaper rash creams were prepared and it was aimed to reach the optimum formulation. All formulations were water/oil type emulsions. In the studies, it was found that the capacity of water/oil type emulsions to carry the oil phase and zinc oxide gave very successful results compared to other systems (22). For this purpose, the most suitable formulation in terms of pH, viscosity, HLB (hydrophilic lipophilic balance) and microbiological quality was revealed. In DR-C-8 formulation, phase separation was observed due to the fact that the emulsifier HLB value was not selected suitable for the oil phase. In DR-C-1/2/3 formulations, it was observed that lanolin was used less and therefore the spread was less. In DR-C-4/5/6 formulations, excessive use of almond oil caused a decrease in the resistance to slipping. In this way, it was thought that it would have a negative effect on the removal of the nappy area, which is a mobile area, and on the elimination of nappy rash. DR-C-7 formulation was selected as the optimum formulation because it is a formulation that does not create high raw material costs in terms of its rheological properties and content. When all parameters between the formulations were compared, DR-C-7 was selected as the optimum formulation containing natural ingredients such as olive oil, lanolin, bees wax, zinc oxide. In our country, the Product Safety Assessment Report is among the documents required by TİTCK during cosmetic product notification and checked by experts. This report presents the evaluation of the finished product, taking into account the toxicological character, chemical structure and exposure levels of the product components, and the specific exposure characteristics of the target group or the area where the product will be applied. Information on the microbiological quality of cosmetic products, verification of the effectiveness of the preservative system and verification of the specified minimum duration of the cosmetic product stored under normal conditions and the duration of use of the finished product after opening are important for product safety. The report must include the results of microbiological quality tests and preservative efficacy tests of the cosmetic product (19). The microbiological analysis of the formulation product obtained in our study was tested and it was concluded that it complied with the microbiological limit values.

CONCLUSION

The antioxidant, antiseptic, anti-inflammatory and antibacterial wound healing purposes of R. damascena, which has phenolic contents such as phenylethyl and flavonoid terpenoids, have been achieved. As a result of the study, no growth was detected in any of the prepared formulations.

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REFERENCES

1. Keskin E, Didisen NA. The Valıdıty And Relıabılıty Study Of The Turkısh Versıon Of The Scale For Assessıng The Severıty Of Uncomplıcated Dıaper Dermatıtıs In Infants. J Basic Clin Health Sci. 2023; 7: 21-29. 9. https://doi.org/10.30621/jbachs.1101510
2. Bante A, Girma M, Ahmed M, Aschalew Z. Diaper dermatitis and associated factors among 1–24 months children visiting public health facilities of Arba Minch town, Gamo zone, southern Ethiopia. Heliyon. 2023; 10 (9): e20785. https://doi.org/10.1016/j.heliyon.2023.e20785
3. Akram M, Ritz M., Munir N, Akhter N, Zafar S, Jabeen F, Ali Shariati M, Akhtar N, Riaz Z, Altaf SH, Daniyal, M, Zahid R, Said Khan F. Chemical constituents, experimental and clinical pharmacology of *Rosa damascena*: A literature review. Journal of Pharmacy and Pharmacology. 2020; 72(2): 161-174. https://doi.org/10.1111/jphp.13185
4. Baydar H, Baydar N G. Yağ Gülü (Rosa damascena Mill.)'nde Distilasyon Ürünlerinin Uçucu Yağ ve Fenolik Madde İçerikleri ile Antiradikal ve Antioksidan Aktiviteleri. 2017; 23:1-9. Journal of Agricultural Sciences
5. Petek TH, Petek M, Petek T, Varda NM. Emerging Links between Microbiome Composition and Skin Immunology in Diaper Dermatitis: A Narrative Review. Children (Basel). 2022; 9(1): 112. https://doi.org/10.3390/children9010112
6. Yildiz I, Kizilca O, Haksayar A, Demirkale ZH. Pediatricians' Knowledge, Attitudes, and Therapeutic Approaches Regarding Diaper Dermatitis: A Common Condition with Many Different Practices. Clinical, Cosmetic and Investigational Dermatology 2023:16 901–910. https://doi.org/10.2147/CCID.S405414
7. Bashir A, Lambert P. Microbiological study of used cosmetic products: highlighting possible impact on consumer health, Journal of Applied Microbiology, 2019; 128(2), 598-605.
8. Rybczyńska-Tkaczyk K, Grenda A, Jakubczyk A, Kiersnowska K, Bik-Małodzińska M.Natural Compounds with Antimicrobial Properties in Cosmetics. Pathogens. 2023; 12(2): 320. https://doi.org/10.3390/pathogens12020320
9. Hoang HT, Moon JY, Lee YC. Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives. *Cosmetics*. 2021;8(4). https://doi.org/10.3390/cosmetics8040106
10. Moser CL, Meyer BK. Comparison of compendial antimicrobial effectiveness tests: A review. *AAPS* PharmSciTech. 2011;12(1):222-226. https://doi.org/10.1208/s12249-010-9575-9
11. Yazıcı EK, Eryilmaz M. Investıgatıon of The Mıcrobıologıcal Contamınatıon Of Used Lıpstıcks And Mascaras. Ankara Universitesi Eczacilik Fakultesi Dergisi. 2023;47(1):269-276. https://doi.org/10.33483/jfpau.1209451
12. Martin A, Busmante P, Chun AHC. 'Coarse Dispersions'. Phyisical Pharmacy. Philedalphia. 1993. s.477-511.
13. Lasseuguette E, Roux D, Nishiyama Y. Rheological properties of microfibrillar suspension of TEMPO-oxidized pulp. Cellulose. 2008; 15, 425-433.
14. Amaral Sobral PJ, Gebremariam G, Drudi F, Saldanha Pinheiro AA, Romani S, Rocculi P. Rheological and Viscoelastic Properties of Chitosan Solutions Prepared with Different Chitosan or Acetic Acid Concentrations. Foods. 2022; 11, 2692. https://doi.org/%2010.3390/foods11172692
15. Lebsing S, Chaiyarit J, Techasatian L. Diaper rashes can indicate systemic conditions other than diaper dermatitis. BMC Dermatology. 2020; 20(1):7. https://doi.org/10.1186/s12895-020-00104-z
16. Pawar KS, Singh PN, Singh SK. Fungal alkaline proteases and their potential applications in different industries. Front. Microbiol. 2023; 14:1138401. https://doi.org/10.3389/fmicb.2023.1138401
17. Wesner E, Vassantachart JM, Jacob SE. Art of prevention: The importance of proper diapering practices. Int J Womens Dermatol. 2019; 5(4): 233–234. https://doi.org/10.1016/j.ijwd.2019.02.005
18. Türkiye İlaç ve Tıbbi Cihaz Kurumu Kozmetik Ürünlerde Güvenlilik Değerlendirmesine İlişkin Kılavuz Sürüm 3.0 (2020). From https://titck.gov.tr/storage/Archive/2020/contentF
19. Angelopoulou P, Giaouris E, Gardikis K. Applications and Prospects of Nanotechnology in Food and Cosmetics Preservation. Nanomaterials (Basel). 2022; 12(7): 1196. https://doi.org/10.3390/nano12071196
20. Kurt, A. A., Aslan, I., & Duman, G. (2021). Next-Generation Natural Baby Barrier Cream Formulations: Physicochemical Analysis and Safety Assessment. Journal of Cosmetic Science, 72(2).
21. Hebert AA. A new therapeutic horizon in diaper dermatitis: Novel agents with novel action. Int J Womens Dermatol. 2021 Sep; 7(4): 466–470. https://doi.org/10.1016/j.ijwd.2021.02.003
22. Tain Y, Zhou J, He C, He L, Li X, Sui H. The Formation, Stabilization and Separation of Oil–Water Emulsions: A Review. Processes 2022; 10, 738. https://doi.org/10.3390/pr10040738