J Immunol Clin Microbiol
Available online at: https://dergipark.org.tr/tr/pub/jicm
DOI: 10.58854/jicm.1402811

Journal of Immunology and Clinical Microbiology

ORIGINAL ARTICLE / ÖZGÜN MAKALE

Developing Natural Shampoo Formulation Against Hair Loss with Microbiologically Tested Herbal Extracts and Essential Oils

Mikrobiyolojik Olarak Test Edilmiş Bitkisel Ekstraktlar ve Esansiyel Yağlar ile Saç Dökülmesine Karşı Doğal Şampuan Formülasyon Geliştirilmesi

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Recieved:10.12.2023, **Accepted:** 17.04.2024

Abstract

Objectives: Shampoo is the most frequently used cosmetic product. Given the importance placed on cleanliness for hair health and the increasing importance placed on hair beauty and care, it is evident that synthetic shampoos damage hair when used regularly. In this context, the objective was to produce a natural shampoo and conduct antimicrobial analysis using *Urtica dioica* (nettle), wheat germ oil, *Rosa damascena Mill* (Isparta rose), and *Allium sativum* (garlic).

Material and Methods: All necessary processes, including formulation design, characterization, and final product tests, were carried out within the scope of the study until the product was packaged and became the final product. Physicochemical tests (pH, viscosity, dandruff effect, microbiological tests) and stability tests (color, odor, appearance, and pH control) were performed on the developed formulation.

Results: The analysis revealed that the pH value of the formulations was approximately 5.5, which is suitable for the skin. The selected NI-S-7 exhibited high viscosity (26.43 ± 0.75), foaming index (250), and high cleaning efficiency among all formulations. This led to developing a shampoo containing active herbal ingredients that nourish and repair hair. Furthermore, the microbiological analysis of the resulting formulation product was conducted and found to comply with the established limit values.

Conclusion: The transformation of agricultural products into commercial products has the potential to bolster the economy. The shampoo in development will be derived from naturally sourced plants cultivated in Turkey and utilized for medical conditions with an aromatherapeutic effect while preserving the typical scent of the rose plant.

Keywords: Herbal Shampoo, *Urtica dioica, Rosa damascena mill, Allium sativum,* Microbiological analysis

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Cite This Article: Ibrahım B, Kurt AA. Developing Natural Shampoo Formulation Against Hair Loss with Microbiologically Tested Herbal Extracts and Essential Oils. *Journal of Immunology and Clinical Microbiology* 2024;9(1):12-23

Öz

Amaç: Şampuan en sık kullanılan kozmetik üründür. Saç sağlığı için temizliğe verilen önem nedeniyle saç güzelliğine ve bakımına verilen önem de giderek artmaktadır. Sentetik şampuanlar düzenli kullanıldığında saçlara zarar verir. Bu kapsamda *Urtica dioica* (ısırgan otu), buğday tohumu yağı, *Rosa damascena Mill* (Isparta gülü) ve *Allium sativum* (sarımsak) kullanılarak doğal bir şampuan üretilmesi ve antimikrobiyal analizlerin yapılması amaçlandı.

Gereç ve Yöntem: Ürün paketlenip nihai ürün haline gelinceye kadar gerekli tüm süreçler (formülasyon tasarımı, karakterizasyon ve son ürün testleri) çalışma kapsamında gerçekleştirildi. Geliştirilen formülasyon üzerinde fizikokimyasal testler (pH, viskozite, kepek etkisi, mikrobiyolojik testler) ve stabilite testleri (renk, koku, görünüm ve pH kontrolü) yapıldı.

Bulgular: Analiz sonucunda formülasyonların pH değerinin cilde uygun olan 5,5'e yakın olduğu belirlendi. Seçilen NI-S-7, tüm formülasyonlar arasında yüksek viskozite (26,43± 0,75), köpüklenme indeksi (250) ve yüksek temizleme verimliliği gösterdi. Saçı besleyen ve onaran bitkisel etken maddeler içeren bir şampuan elde edildi. Ayrıca ortaya çıkan formülasyon ürününün mikrobiyolojik analizi yapılmış ve Türkiye İlaç ve Tibbi Cihaz Kurumu (TITCK) tarafından yayınlanan kozmetik ürünlerde güvenlilik kılavuzına göre sınır değerlere uygun olduğu görülmüştür.

Sonuç: Ülkemizde yetiştirilen ürünlerin ticari ürüne dönüştürülmesi ekonomiye destek olacaktır. Geliştirilecek şampuan, gül bitkisinin tipik kokusunu koruduğu için Türkiye'de yetişen doğal kaynaklı bitkilerden elde edilecek ve aromaterapi etkisiyle tıbbi rahatsızlıklarda kullanılması beklenmektedir.

Anahtar Kelimeler: Bitkisel Şampuan, *Urtica dioica, Rosa damascena mill, Allium sativum,* Mikrobiyolojik analiz

INTRODUCTION

As the economy improves and people become more interested in beauty, the demand for health and skincare products also increases. For centuries, herbs have been popular for these purposes. Cosmetics and foods that contain new bioactive ingredients for skincare and beauty are constantly being researched and developed. The skin is affected by several factors such as ultraviolet rays, stress, hormones, and aging, which can cause loss of elasticity, changes in pigmentation, and wrinkles. Many medicinal plants have been shown to have beneficial effects on the skin, with *Rosa damascena* Mill, *Urtica dioica* L, and *Triticum vulgare* being among the most notable (1,2,3). Research in the field of cosmetology has proven the effect of Rosa **damascena** on rehydrating the skin, reducing scars and stretch marks, managing acne, reducing skin pigmentation, and delaying wrinkles. It is recommended as a skin-revitalizing agent (1). It has been determined that these products may be contaminated with microorganisms found in the environment during production (4). Shampoos are one of the most widely used cosmetic products. To have healthy hair, herbal sources are used as active ingredients to nourish and protect the hair. However, they can be used for different needs formulations that contain high amounts of synthetic chemicals, which can cause a lot of damage to the hair with regular use (5). Aqueous cosmetic products provide a suitable environment for the growth of fungi

and bacteria due to the moisture they contain. Microbiological analysis is carried out on the shampoo for this particular purpose (6). *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Aspergillus spp* are microorganisms that frequently cause contamination in cosmetic products. Water and organic/ inorganic compounds are susceptible to microbial contamination under appropriate physicochemical conditions, including those encountered in the production of cosmetics (7,8). To prevent microbial growth, various substances with different chemical structures are added to protect against contamination (9,10). The objective of this study was to develop eight distinct natural herbal hair shampoo formulations with the potential to prevent hair loss and dandruff. The formulations were created using extracts and oils of *Rosa damascena*, *Urtica dioica*, and *Triticum Vulgare* (Wheat), which are native plants of Turkey. Following the completion of the characterization and safety evaluation, it was determined that the AHL-NS-7 shampoo formulation exhibited the most promising characteristics among the eight formulations. The formulation exhibited no stability issues, rendering it the most secure option for consumers.

MATERIAL AND METHODS

Material

The composition of the product includes *Rosa damascena* Extract (Talya), Urtica dioica Leaf Extract (Talya), *Triticum vulgare* (Wheat) Germ Oil (Talya), Guar Gum (BASF), Ammonium Lauryl Sulfate (Sabunaria), Cocamide MEA (Sabunaria), Betaine (Sabunaria). Additionally, the product contains Ethylhexylglycerin (Ashland), Laureth-7 Citrate (BASF), and all other ingredients of cosmetic quality. The following instruments were employed: a Milwaukee MW150 max (Szeged, Hungary), a rotational viscometer PCE-RVI 10 (Meschede, Germany), a mechanical mixer (Isolab, Germany), an incubator Elektromag M5040 PS (Cerkezkoy, Turkey), and an Ultraturrax (VELP Scientifica, Italy).

Methods

A formulation was developed through polymer hydration and high-speed homogenization at elevated temperatures to enhance the performance of shampoos. Physicochemical tests (pH, viscosity, dandruff effect, microbiological tests) and stability tests (color, odor, appearance, and pH control) were conducted on the developed formulation. The pour plate method was employed to conduct total bacterial and fungal analyses for *Staphylococcus aureus*, aerobic mesophilic bacteria and yeast mold, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, with the results expressed in colony-forming units per milliliter (CFU/ml). By the established standards, the pathogens that should not be present in 1 **g** or 1 ml of the cosmetic product were identified as *E. coli*, S. *aureus*, *P. aeruginosa*, and *C. albicans* (ISO 17516, 2014). Selective media were employed to detect the pathogens.

Microbiological Analysis

The formulation developed for microbiological analysis was disinfected with 70% ethanol within the scope of the study. To dissolve the product, 5 g/L polysorbate 80 was added to 90 ml TSP (Buffered Sodium Chloride Peptone), and 10 g of sample was added and left to dissolve in a water bath for 10-15 minutes. Serial dilutions (10-2, 10-3, and 10-4) were prepared by transferring 1

ml of the sample suspension into 9 ml of TSP using the pour plate method. One milliliter was then taken from the dilution tubes and transferred to a 90 mm petri dish, which was repeated twice. Subsequently, 15-17 ml of agar medium, cooled to 45°C in a water bath, was poured into petri dishes and allowed to solidify. The total count of aerobic mesophilic microorganisms was determined using Tyriptic Soy Agar (TSA) at 30-35°C for 3-5 days. For total yeast and mold counting, SDA medium was employed, with the medium incubated at 20-25°C for 5-7 days. In the case of breeding, the calculation formula is used to count colonies visible to the naked eye. This formula is as follows: The number of Colony Forming Units per milliliter (CFU/ml) is calculated by multiplying the total number of colonies obtained by the dilution factor and then dividing by the sample volume.

Enrichment

A 10 g sample was dissolved in a buffered peptone solution and transferred to a 90 ml Tryptic Soy Broth (TSB) medium. This medium contains lecithin and polysorbate, which are necessary for neutralization and are suitable for use as a general growth medium. After thorough shaking, the sample was incubated at 30-35°C for 18-24 hours (up to a maximum of 72 hours). After incubation, a selective medium was used. Enrichment for *E. coli*, *P. aeruginosa*, and *S. aureus* was applied. For *C. albicans*, 10 ml (1 g or ml) of the sample dissolved in TSP was taken and transferred to a 90 ml Sabouraud Dextrose Broth (SDB) medium. After shaking well, it was incubated at 30-35°C for 72 hours (maximum five days). Subsequently, a selective medium was employed for incubation.

Investigation of Aerobic Mesophilic Bacteria

Following enrichment, 1 ml of TSB medium was transferred to sterile petri dishes. Five milliliters of the medium were added to Tryptone Glucose Extract Agar (TGEA) medium cooled to 45 °C, mixed, and inoculated with the sample in duplicate. The inoculated medium was then allowed to solidify. After solidification, the inoculated medium was incubated at 37 °C for 48 hours. In the event of reproduction after the incubation period, the number of colonies formed is calculated by taking the dilution factor into account.

Investigating The Presence of Escherichia coli

Following enrichment, 1 ml of TSB medium was transferred to sterile petri dishes. Thereafter, 5 ml of MacConkey Agar (MCA) medium was inoculated with the sample in duplicate and allowed to solidify. The inoculated plates were incubated at 30-35°C for 24 hours (up to a maximum of 48 hours).

Investigating The Presence of Staphylococcus aureus

Following enrichment, 1 ml of TSB medium was transferred to sterile petri dishes. Five milliliters of cooled (to 45 °C) Mannitol Salt Agar (MSA) medium was inoculated with the sample in duplicate and allowed to solidify. The inoculated MSA was incubated at 30-35 °C for 24 hours (with a maximum incubation period of 48 hours).

Investigating The Presence of Pseudomonas aeruginosa

Following enrichment, 1 ml of TSB medium was transferred to sterile petri dishes. Five milliliters of medium was added to the Cetrimide Agar (CA) medium that had been cooled to 45 °C, and mixed, and the medium

was inoculated with the sample in duplicate. The inoculated medium was then allowed to solidify. The plates were incubated at 25 °C for 5 to 7 days.

Formulation Study

Guar gum was combined with two-thirds of the water at 400 rpm for five minutes. A citric acid solution was then added under stirring. Surfactants and foaming agents were subsequently incorporated into the mixture. Herbal extracts and essential oils were dissolved in Laureth-7 Citrate at varying concentrations and added to the mixture under 400 rpm. The pH value of the mixture was then regulated with 1M NaOH. Consequently, eight distinct formulations with varying consistencies and active ingredients were developed (Table 1, 2).

Table 1. Components and concentrations of shampoo formulations

Contents	BSF-1
Rosa damascena Extract	0.5-1
Urtica Dioica Leaf Extract	0.2-0.4
Allium Sativum Bulb Oil.	0.2
Triticum Vulgare (Wheat) Germ Oil	0.2-0.4
Guar Gum	0.1-0.9
Amonyum Lauril Sulfate	5-6.5
Amonyum Lauril Eter Sulfate	6-7.5
Cocoamide Mea	4-5.5
Betain	5-6.5
Dimethicone	1-3
Glycerine (Humectant)	1-3
Laureth-7 Citrate	1
Ethylene Glycol Distearate	0.5
Butyl Hydroxy Toluene	0.05
EDTA (Etilendiamin tetraase-tik asit)	0.3
Evans	0.01
Water	k.m.

Table 2. Ingredients and quantities used for eight different shampoo formulations

Ingredients	**NI-S-1**	**NI-S-2**	**NI-S-3**	**NI-S-4**	**NI-S-5**	**NI-S-6**	**NI-S-7**	**NI-S-8**
Rosa Damascena Extract	0.5	0.5	0.5	0.5	1	1	1	1
Urtica Dioica Leaf Extract	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4
Allium Sativum Bulb Oil.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Triticum Vulgare (Wheat) Germ Oil	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4
Guar Gum	0.1	0.3	0.6	0.9	0.1	0.3	0.6	0.9
Amonyum Lauril Sülfat	5	5.5	6	6.5	5	5.5	6	6.5
Amonyum Lauril Eter Sülfat	6	6.5	7	7.5	6	6.5	7	7.5
Cocoamide Mea	4	4.5	5	5.5	4	4.5	5	5.5
Betain	5	5.5	6	6.5	5	5.5	6	6.5
Dimethicone	1	2	3	4	1	2	3	4
Glycerine (Humectant)	1	2	3	4	1	2	3	4
Laureth-7 Citrate	1	1	1	1	1	1	1	1
Ethylene Glycol Distearate	0.5	0.5	0.5	0.5	1	1	1	1
Butyl Hydroxy Toluene	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
EDTA	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Essence	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	74.95	70.95	66.65	62.35	73.75	69.55	65.25	60.95

Control of Physicochemical Properties

Following the shampoo's retention in a standard room environment, its visual characteristics, including color, were evaluated.

Foaming Power

The cylinder shaking method was employed to assess the foaming capacity of the sample. Five milliliters of the solution was extracted from the shampoo and placed in a 25-ml graduated cylinder. The cylinder's opening was sealed manually and the sample was agitated for 10 seconds. After that, 1-5 ml were transferred to 5 distinct tubes at varying concentrations and the tubes were closed and agitated for 10 seconds. The total volume of the foam contents was recorded 1 minute after shaking. The foam volume was calculated exclusively. Following the initial shaking, the foam volume was recorded in centimeters at one-minute intervals for four minutes, and the foaming index was calculated (11). This process was repeated three times and documented.

Wetting Strength

The wetting ability of a solution is a function of its concentration. For an effective solution, the wetting time should be as low as possible. In other words, the less the wetting time, the more efficient the solution. Table 2 shows that the soaking time of all three shampoos is minimal, indicating that they are of good quality.

Cleaning Power Test

Two drops of shampoo are added to 10 mL of water, and one drop of ink is added to the test tubes. The mixture is then shaken 10 times. The amount of ink present in the foam is rated as none, light, medium, or heavy.

Rheological Studies

The apparent viscosity was determined by using a PCE-RVI-10 rotational viscometer (PCE Instruments, Hamburg, Germany). The 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 was measured with an L2 spindle at 6 rpm for 120 sec throughout. The slip rate is calculated by the rotation speed and the gap between the shaft and the container (Equation X). 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. The sliding speed was calculated according to the formulation x.

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

In the equation, "y" indicates the sliding speed in s-1, "Ni" indicates the rotation speed in rpm, and "R0" and "Ri" indicate the radius of the bowl and shaft in mm. Apparent viscosity values were plotted as a function of shear rate and fitted into Equation x according to the Ostwald-de Waele relationship (13).

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

In this context, K represents the viscosity coefficient, while n is the flow behavior index, which is calculated from exponential regression (14).

Calculation of the interval of safety (MoS)

The systemic exposure dose (MOS) of the shampoo formulation was taken as 10.46 g/day, with application frequency (1/ day) and daily exposure level according to

skin surface area (1440 cm2) and product type, as specified in cosmetic guideline 3. Systemic exposure dose was calculated with the formula x and safety margin values were calculated with the formula x. Looking at the results, the exposure doses and MoS values of the components in all formulations were found to be>100.

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

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

Stability Study

Stability studies were carried out to evaluate the product's appearance, color, pH, viscosity, and microbial growth in three different conditions: refrigerated, at room temperature, and in an oven set at 45°C for six months. These studies were conducted following the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) directives and TITCK cosmetic regulations.

RESULTS

Microorganism Analysis Result

The shampoo product was tested and no growth was observed. Table 1 shows that no growth was observed on the 14th and 28th days following the effect of the preservative.

Table 3. 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

Table 4 presents the rheological properties, foaming index, cleaning effect, color, and appearance results of the formulations with a skin pH value of 5.5 suitable for the scalp.

Table 4. Appearance, pH, texture, foam index, and rheological results of the formulations (n=3).

Formulation code	Appearance	Color	Texture	Foaming index	Direct Dispersion Test	Ph Measurement Result	Viscosity (Pa.s)	Glide Speed (1/sec)	Glide Voltage (D/cm²)
NI-S-1	Homojen	Opaque-White	Smooth	500	Light	5.612±0.12	12.89±0.30	0.11	117.15±2.77
NI-S-2	Homojen	Opaque-White	Smooth	250	Light	5.343±0.13	14.39±0.36	0.11	130.82±3.27
NI-S-3	Homojen	Opaque-White	Smooth	250	None	5.435±0.11	23.30± 0.90	0.12	194.17±7.52
NI-S-4	Homojen	Opaque-White	Smooth	125	None	5.342±0.10	35.39±0.65	0.11	321.70±5.93
NI-S-5	Homojen	Opaque-White	Smooth	500	Light	5.453± 0.09	13.15±0.18	0.12	109.58±1.47
NI-S-6	Homojen	Opaque-White	Smooth	250	Light	5.498± 0.11	15.19±0.28	0.13	116.87±2.81
NI-S-7	Homojen	Opaque-White	Smooth	250	None	5.567± 0.12	26.43± 0.75	0.12	220.22±6.26
NI-S-8	Homojen	Opaque-White	Smooth	125	None	5.601± 0.12	37.32±0.77	0.13	287.08±5.89

Rheological Study Result

The anti-dandruff and hair-strengthening natural shampoo formulation demonstrated shear thickening according to the Ostwald-de Waele model and exhibited pseudo-plastic flow. The results indicate that the shear rate is between 0.15 and 30.59 s-1, the shear stress is between 32.58 and 249.34 (D/cm2), and the viscosity values are between 213 and 81.50 Pa.s (Table 5, Fig. 1).

Table 5. Rheological properties of optimum formulation NI-S-7

Viscosity (Pa.s)	Glide Speed (1/sec)	Glide Voltage (D/cm²)
215.00±5.86	0.15	33.06±0.85
134.00±7.81	0.31	40.08±0.81
90.57±6.06	0.76	69.16±0.06
54.77±5.53	1.53	80.80±4.30
38.43±4.41	3.06	118.44±13.33
25.03±1.50	6.12	155.69±6.78
13.83±3.06	15.30	216.85±22.66
8.12±1.41	30.59	247.99±9.18

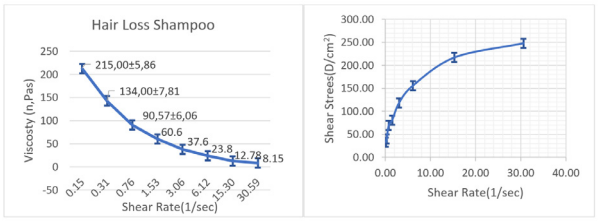

Figure 1. Viscosity results versus shear rate for NI-S-6 (n=3)

Confidence Interval Results

Upon examination of the results. it was determined that the exposure doses and MoS values of the components in all formulations were greater than 100.

Stability Results

The physicochemical properties of shampoo formulations prepared with extracts obtained from natural herbal sources were found to be preserved under all stability conditions. Following organoleptic controls, it was observed that the specific appearance and color remained unchanged from day 0 to 6 months. It was also observed that the pH value was within the range of pH 5.3-5.8 under all conditions, which is compatible with the acceptable pH value for the skin. The viscosity of the shampoo formulation was monitored over time at different temperatures. The viscosity values were found to vary between 23,800 and 26,382 (Pa.s). To control microbiological growth, a protective effectiveness test (45°C) was carried out in an oven, and no growth was detected (Table 6).

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

Stabılıty Condıtıon	Stabılıty Condıtıon	Appearance	Colour	Smell	Mıcrobıologıcal Growth In The Oven (45°C)
Room Conditions	0 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	3 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	6 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
In the oven (45°C)	0 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	3 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	6 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
Refrigerator	0 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	3 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth
	6 st month	Homogeneous-Cream	Specıfic	Specıfic	NO growth

DISCUSSION

In recent years, shampoo formulations have focused on products with functional features, such as strengthening hair, preventing dandruff, and accelerating hair growth. However, research indicates that products are frequently contaminated during consumer use (15). The use of cosmetic products without consideration of their expiration date, the sharing of products with multiple individuals, the insertion of contaminated fingers, and the wetting of products with saliva are among the causes of contamination (16). It has been established that cosmetic products with a high water content are more susceptible to microbial growth if contaminated during use (17,18).

A review of the literature reveals that several microorganisms commonly found in cosmetic products include *P. aeruginosa*, *S. aureus*, *Enterobacter spp.*, *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Candida albicans*, and *Aspergillus spp.* Some microorganisms that cause contamination metabolize some substances in the product with their hydrolytic enzymes. As a result, various changes may occur gas formation, viscosity and color changes, taste changes, turbidity, precipitation, and membrane formation (19,20,21).

Intact skin and mucous membranes serve as a physical barrier for microorganisms. Damage to these structures and disruption of their integrity for various reasons allow microorganisms to enter the body. It is well documented that many microorganism species detected as contaminants in cosmetic products are pathogenic and can cause serious infections (19,22). *Pseudomonas aeruginosa* is a microorganism that is frequently implicated in the contamination of cosmetics. It is widely distributed in the

natural environment, particularly in soil and water. As an opportunistic pathogen, it can cause serious infections, including those of the respiratory, urinary, wound, and burn tracts, meningitis, and septicemia. These infections are particularly prevalent in individuals with immunodeficiency. Pseudomonas aeruginosa is among the nosocomial infection factors for which treatment is challenging due to its ability to rapidly develop resistance to antimicrobials (22). It is imperative that cosmetics, which are non-sterile pharmaceutical products, be delivered to consumers in an effective, safe, and high-quality manner. This is guaranteed in our country by the legislation published by TITCK. Contamination of cosmetic products with microorganisms can have detrimental effects on consumer health and cause significant economic losses. Research indicates that cosmetic products are often exposed to contamination during use by the consumer. The most significant causes of contamination during use are the use of products after the stated expiration date, use by multiple individuals, insertion of fingers or contaminated objects into the product, and contact with air. In a study, different cosmetic product groups were evaluated in terms of microbiological contamination. The first group was used by a single individual for its usage period, which had not yet expired. The second group was used by more than one individual for its usage period, which had not yet expired. The third group was used after the expiration date. The results indicated that the second group exhibited a higher contamination rate than the first group, and the third group exhibited a higher contamination rate than both groups (17, 23, 24). In the European Union, green agreement processes and sustainability studies tend to favor the use of natural ingredients in the preparation of formulations. Among the eight different formulations prepared in this study, the NI-S-7 formulation is particularly noteworthy in that it meets these needs, containing *Rosa damascena* Mill, *Urtica dioica* L, and *Triticum vulgare*. Furthermore, an examination of the formulation's physicochemical properties revealed that an increase in the concentration of guar gum directly affected viscosity, preventing fluidity from occurring in the bottle. Formulations NI-S-1, NI-S-4, NI-S-5, and NI-S-8 were eliminated because they did not have the desired flow properties. It was observed that the active ingredient was high in the formulations NI-S-2, NI-S-3, NI-S-6, and NI-S-7, which were suitable in terms of flow properties. However, the NI-S-7 formulation was thought to be most suitable due to its low Guar gum content and acceptable flow properties. All formulations were found to be suitable for application in terms of pH values. The strongest formulations in terms of foam index were identified as NI-S-4 and NI-S-8. The remaining formulations, in descending order of foam strength, were NI-S-2, NI-S-3, NI-S-6, and NI-S-7. The formulations with the least foam strength were identified as NI-S-1 and NI-S-5. The NI-S-7 formulation was found to be a suitable foam index, as the surfactants used exhibited a low index value, which was deemed acceptable.

A comparison of the cleaning properties of the two formulations, NI-S-7 and NI-S-6, revealed that NI-S-7 was the more effective of the two. Consequently, NI-S-7 was selected as the optimal formulation for microbiological stability.

CONCLUSION

The transformation of products grown in our country into commercial products will support the economy. The shampoo to be developed will be obtained from naturally sourced plants grown in Turkey and can be sold in pharmacies for medical disorders with its aromatherapy effect as it preserves the typical scent of the rose plant. To prevent microbiological contamination in cosmetic products, microbiological controls must be carried out starting from the raw material, and production must be carried out under hygienic conditions by Good manufacturing practice (GMP) rules. The legislation stipulates that preservatives should be added to products at risk of contamination, which can protect the product in a way equivalent to the expected shelf life plus the period of use. The preservatives should have a broad spectrum of action and not have any allergic, toxic, or irritating effects on the consumer. After production, the product's shelf life and usage time after opening should be determined through stability tests.

ACKNOWLEDGEMENT

I would like to thank Dr Ahmet Arif Kurt for her support in the study.

Ethical Declaration: Not applicable

Financial Support: Non

Conflict of Interest: There is no conflict of interest

Authorship Contributions: Concept: BI, AK, Design: BI, AK, Data collection: BI, AK, Analysis and interpretation: BI, Literature search: BI

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