Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Development of natural acne mask formulations from rose pomace waste

Ahmet Arif KURT[1,2], Bashar IBRAHIM[3*]

1 Department of Pharmaceutical Technology, Faculty of Pharmacy, Süleyman Demirel University, Centre 32200 Isparta/Turkey
2 Research Laboratory Department, Polisome R&D Pharmaceutical Industry Trade Co., Centre 32200 Isparta/Turkey
3 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Süleyman Demirel University, Centre 32200 Isparta/Turkey

* Corresponding Author. E-mail: basharibrahim@sdu.edu.tr (B.I.); Tel. +09-0534-335-61-99.

Received: 24 July 2024/ Revised: 23 September 2024 / Accepted: 23 September 2024

ABSTRACT: The rose plant has been used by different societies throughout human history for beauty and health purposes. *Rosa damascena* Mill., *R. gallica* L., and *R. phoenicia* Boiss belong to the Rosaceae family and are hybrid plants of different species. The study aims to develop an acne mask containing natural ingredients using the rose pulp of the *Rosa damascena* plant produced in the Isparta region and released into the environment as waste. The sample was prepared using the Milestone ETHOS ONE microwave unit. The sample included the addition of nitric acid and hydrogen peroxide solution and the use of the wet digestion method and was analyzed using the pour plate method. The rose mask formulation consists of rose pulp, thermal water, clay, glycerin, aloe vera, hyaluronic acid, preservative, EDTA, and sodium gluconate. The research led to a mask formulation that maintains absorption, moisturizing, and renewal properties. The two formulations that exhibited the fastest drying times were GP-AMF-10 and GP-AMF-8, with drying times of 10.21 and 10.40, respectively. Microbiological analysis of the resulting formulation product was conducted and it was determined to comply with microbiological limit values. After a comprehensive evaluation, it was found that the selected mask formulation demonstrated superior characteristics..

KEYWORDS: Cosmetics; Facial Mask; Rose pulp; Acne; Microbiological analysis.

1. INTRODUCTION

Acne is a chronic, inflammatory disease of the pilosebaceous unit that affects 9.4% of the world's population. It is the most common skin problem in teenagers. It is a normal part of puberty [1]. In vitro experiments have demonstrated that Propionibacterium acnes phylotypes can elicit disparate inflammatory reaction patterns. Specific phylotypes have been observed to induce particular acne lesions and exhibit varying degrees of resistance to antimicrobials [2]. In recent years, significant advancements have been made in our understanding of the underlying mechanisms of acne, particularly inflammatory processes. This has led to the development of novel treatment approaches. While topical agents may be effective in mild acne cases, they are often inadequate in moderate to severe acne [3]. The causes of acne are multifactorial, including genetic predisposition, dietary habits, frequency of skin cleansing, and air pollution. The use of cosmetic products by individuals of almost every age group in society has a long historical precedent [4].

The distribution of plant communities in our country has been affected by changes in climate. As a consequence of ecological and phytogeographic differentiation, our country is endowed with a considerable number of endemic species. In comparison to the 2,500 endemic plant species present in Europe, Turkey boasts 3,000 endemic species [5]. It is notable that species originating from diverse geographical regions are cultivated in locations that are strikingly disparate. The World Health Organization (WHO) has indicated that 60% of the global population relies on plants to maintain general health [6]. In recent years, the discovery of antibiotic-resistant strains and the emergence of side effects seen in synthetic drugs have prompted scientists to conduct research with the aim of obtaining similar effects from natural sources [7]. A multitude of medicinal plants have been identified by numerous scientists, and the effects of the majority of

How to cite this article: Kurt AA, Ibrahim B. Development of natural acne mask formulations from rose pomace waste . J Res Pharm. 2025; 29(1): 1-10.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1626199

them have been validated Medicinal and aromatic plants are defined as those cultivated for their bioactive substances [8]. The significance of these plants is largely determined by the bioactive substances they contain. Our country boasts a rich and diverse flora, particularly in terms of plants that produce essential oils. Among these, the rose plant is notable for its prominence in the commercial essential oil industry within our country [9].

The treatment of sebum deficiencies and the reduction of inflammation in acne lesions are achieved through the utilization of *Rosa damascena* extract. The residue resulting from the distillation of the rose petals is a byproduct that is ultimately discarded into the environment [10]. Studies have shown that rose oil has a rich phenolic substance content, and pulp water contains more phenolic compounds than rose water [6]. Flavonols are compounds that possess exceptionally high antioxidant properties. The quantity of flavonoids present in rose pulp is nearly equivalent to that found in rose oil, and this concentration is considerably higher than that observed in rose water [11]. A review of the literature reveals that rose essential oil has a multitude of beneficial effects, including antibacterial, antiviral, antifungal, antiseptic, antioxidant, analgesic, anti-inflammatory, and wound-healing properties [12]. It is estimated that approximately 15,000 tons of rose flowers are produced globally on an annual basis. Approximately 8,500 tons of this production is manufactured in Turkey, with Bulgaria accounting for an additional 7,000 tons. Rose oil and rose concrete produced in both countries are primarily utilized as commercial materials in the global perfume, cosmetics, and pharmaceutical industries. Additionally, partial production occurs in Morocco, Iran, Afghanistan, China, India, select Caucasian countries, Saudi Arabia, and certain Northern Block countries, with the precise production quantities remaining uncertain. Furthermore, the production of one kilogram of rose oil necessitates the processing of 3.5 to 4.5 tons of rose petals, resulting in the generation of a considerable quantity of rose pulp, which is subsequently released into the environment [13]. To ascertain whether the optimum formulation presents any potential harm to users, it is essential to calculate the MoS values within the safety limit [14]. Microorganisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus* species are known to frequently cause contamination in cosmetic products [15].

Today, protective effectiveness tests are carried out according to different methods determined by institutions such as the United States Pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP), and American Society for Testing and Materials (ASTM) [16]. These products must undergo a rigorous microbiological evaluation before being made available to the market, to ensure the safety and well-being of the intended audience. Several methods can be employed for microbiological analysis, including pouring, spreading, and dripping [17] The principle of these methods is that the microorganism forms a colony in the solid environment of the microorganisms in the sample.

Our study, which aims to bring this rose pulp released into the environment in the Isparta region into the economy with zero waste application, aimed to develop an acne mask containing natural ingredients from the Rosa Damascena plant.

2. RESULTS

The research findings have led to the development of a mask with the following characteristics: rapid absorption, deep moisturizing, skin renewal, and formulations that can be evenly distributed to the application area with fingers. Additionally, the formulations are designed to remain in the application area and not flow. Furthermore, the resulting formulation product underwent microbiological analysis, which demonstrated compliance with the established limit values.

2.1. Physicochemical test results

The physicochemical properties of acne mask formulations were investigated through the measurement of pH, the assessment of spreadability, the determination of drying time, and the evaluation of film formation performance. The pH measurements of the face mask formulations exhibited a range of 5.013 ± 0.200 to 5.335 ± 0.190. These values are consistent with the pH range of 5.0 to 6.0, which is typical of the pH of the facial area. Upon examination, the mask formulations were found to exhibit excellent spreadability and cleansing properties on the skin. A comparison of the drying times of the formulations revealed that an increase in the ratio of powder components (kaolin, talc, thermal clay) resulted in a corresponding increase in the drying time. The two formulations that exhibited the fastest drying times were GP-AMF-10 and GP-AMF-8, with drying times of 10.21 and 10.40, respectively. The evaluation of film formation performance

revealed that the mixture of powder components in the formulations containing hyaluronic acid and aloe vera was effective in facilitating the formation of a film. It was observed that the film formation performance of the formulations in which the powder mixture constituted 40% was optimal (Table 1, Figure 1).

Table 1. Physicochemical test results

Formulations	pH mesurmnet result (n=3)	Spreadability assessment	Drying Time	Film Making Performance
GP-AMF-1	5.220 ± 0,210	Spreadability good	12.50 sc	Film formation is poor
GP-AMF-2	5.116 ± 0,200	Spreadability good	13.42 sc	Film formation is poor
GP-AMF-3	5.135 ± 0,220	Spreadability good	13.15 sc	Film formation is poor
GP-AMF-4	5.095 ± 0,215	Spreadability good	13.32 sc	Film formation is poor
GP-AMF-5	5.274 ± 0,270	Spreadability good	11.13 sc	Film formation is good
GP-AMF-6	5.335 ± 0,190	Spreadability good	11.48 sc	Film formation is good
GP-AMF-7	5.280 ± 0,250	Spreadability good	11.45 sc	Film formation is good
GP-AMF-8	5.013 ± 0,200	Spreadability good	10.40 sc	Film formation is good
GP-AMF-9	5.088 ± 0,190	Spreadability good	12.10 sc	Film formation is poor
GP-AMF-10	5.123 ± 0,210	Spreadability good	10.21 sc	Film formation is good

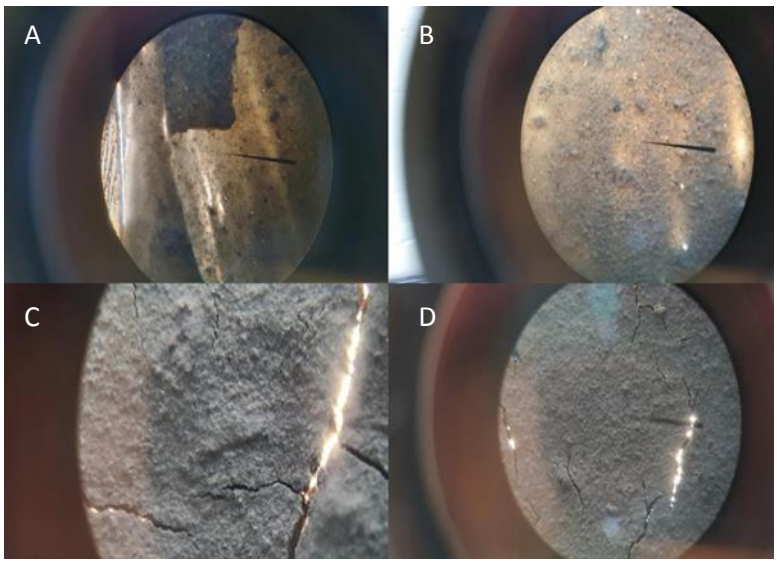

Figure 1. Microscope images of masks (A: Formulation 10, B: Formulation 8, C: Formulation 2, D: Formulation 9) analysed at 10x10 magnification

2.2. Rheological Studies

The formulations of acne masks resulted in n<1 and exhibited Dilataneous flow, showing shear thickening according to the Ostwald-de Waele equation. According to the results obtained, the optimum formulation GP-AMF-8 shear rate is between 0.15 and 30,59 s^{-1}, shear stress is between 15,30±5,49 and 81382,36±6,32 (D/cm²), and viscosity values are 10000±5,89 and 266000±5,63 cPs (Table 1,2.- Figure 2).

Table 2. Viscosity measurement results of formulations

Formulations	Torque (%, n=3)	Viscosty (cPs, n=3)
GP-AMF-1	45.80 ± 2.3	91600 ± 1.8
GP-AMF-2	57.00 ±3.1	114000 ± 2.7
GP-AMF-3	42.40 ± 2.6	84800 ± 2.3
GP-AMF-4	48.10 ± 1.9	96200 ± 1.2
GP-AMF-5	40.10 ± 2.1	80200 ± 1.9
GP-AMF-6	57.60 ± 1.3	57600 ± 3.1
GP-AMF-7	55.80 ± 1.8	95600 ± 2.8
GP-AMF-8	51.30 ±0.9	51300 ±1.1
GP-AMF-9	40.70 ± 2.2	81400 ±3.4
GP-AMF-10	49.10 ±1.3	4910 ± 2.2

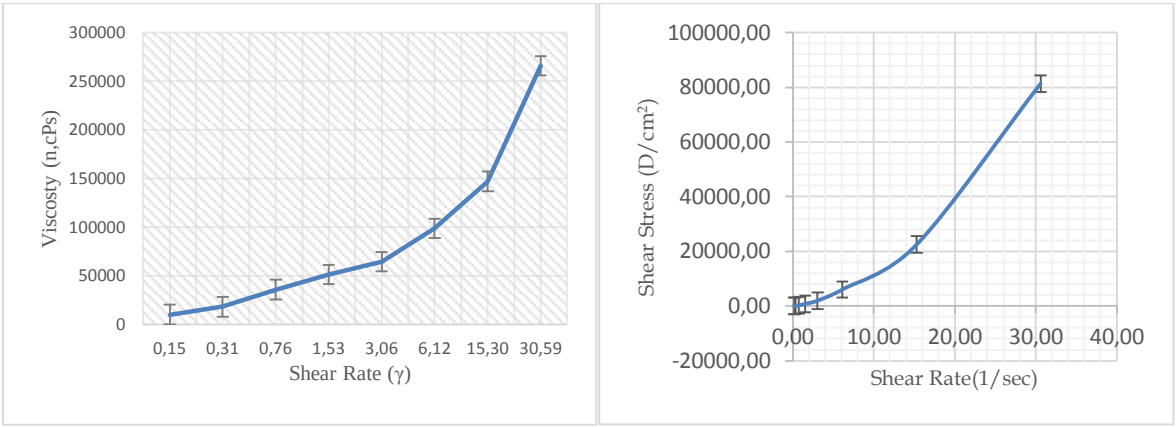

Figure 2. Viscosity values versus shear rate and and shear rate versus shear stress of GP-AMF-8

2.3. Products Safety Assessment

In consideration of the findings about the safety interval, the exposure doses, and the MoS values of the components across all formulations were determined to be more than 100 (>100).

2.4. Stability Results

The rose pulp face mask demonstrated consistent physicochemical properties across all testing environments. Following controls, it was noted that the product's specific appearance and color remained consistent from day 0 to 6 months. The pH value was found to be within the range of 5.3-5.8 under all conditions, which is in line with the acceptable pH range for use on the skin. The viscosity of the face mask was monitored over time at varying temperatures, and the resulting viscosity values are presented in Table 3. The concentration of substances in the composition of the optimum formulation (GP-AMF-8) is appropriate according to the safety assessment results (Table 4). The face mask formulation we tested was observed to be free of microbial growth (Table 5,6).

Table 3. Viscosity measurement results of the optimum formulation (GP-AMF-8)

Viscosty (cPs)	Rotation Speed (rpm)	Torque (%)	Shear Stress (D/cm²)	Shear Rate (1/sec)	Temp. (0C)	Time Range (mm:ss.t)
10000±5.89	0.3	100	15.30±5.49	0.15	25°C	00:02:00
18080±8.93	0.6	90.4	55.32±7.62	0.31	25°C	00:02:00
35750±7.56	1.5	73.50	273.44±6.36	0.76	25°C	00:02:00
51300±4.34	3	51.30	784.76±5.54	1.53	25°C	00:02:00
64600±9.78	6	32.30	1976.43±6.21	3.06	25°C	00:02:00
98800±6.57	12	29.70	6045.55±5.80	6.12	25°C	00:02:00
147000±8.50	30	14.70	22487.23±8.65	15.30	25°C	00:02:00
266000±5.63	60	13.30	81382.36±6.32	30.59	25°C	00:02:00

Table 4. Safety Assessment of Formulation GP-AMF-8

Ingredients	PODsys(mg/kg)	MOS	MOS
Rose Pump	1000	13793.1>100	13793.10
Thermal Clay	1600	315.27>100	315.27
Talck	1600	1471.5>100	1471.26
Kaolin	600	551.72>100	551.72
Glycerin	200	1379.31>100	1379.31
Propylene Glycol	10400	71724.14>100	71724.14
Aloe Vera	1845	25448.28>100	25448.28
Hyaluronic Acid	250	8620.69>100	8620.69
Ethylhexylglycerin	800	55172.41>100	55172.41
Phenoxyethanol	500	6896.55>100	6896.55
EDTA	250	17241.38>100	17241.38
Thermal Water	35000	5224.66>100	5224.66

Table 5. Stability results; appearance, colour, odour, viscosity, pH and microbiological growth results as of Day 0, Month 3 and Month 6

Stability condition	Control period	Appearance	Color and smell	Microbiological control
Room	0th Month	Homogeneous	Specific	No growth
	3th Month	Homogeneous	Specific	No growth
	6th Month	Homogeneous	Specific	No growth
İncubator (45°C)	0th Month	Homogeneous	Specific	No growth
	3th Month	Homogeneous	Specific	No growth
	6th Month	Homogeneous	Specific	No growth
Refrigerator	0th Month	Homogeneous	Specific	No growth
	3th Month	Homogeneous	Specific	No growth
	6th Month	Homogeneous	Specific	No growth

Table 6. The results of the microbiological tests

Microorganisms	Conclusion
Total number of aerobic mesophilic microorganisms (Bacteria, mold, yeast)	The product contains no more than 1 x 10^2 colony-forming units (CFU) per gram.
Yeast and mold	The product contains no more than 1 x 10^2 colony-forming units (CFU) per gram.
Escherichia coli	No growth
Pseudomonas aeruginosa	No growth
Staphylococcus aureus	No growth
Candida albicans	No growth

3. DISCUSSION

The genus Rosa, commonly known as the rose, is one of the most significant plants in horticulture and industry, comprising approximately 200 species and over 18,000 varieties. Turkey is home to approximately 50 distinct rose varieties, 24 of which are native to the region. The rose is a ubiquitous plant in Turkey, occurring in nearly every region of the country. *Rosa damascena* Mill, also known as the Isparta rose, oil rose, or simply oil rose, is a species of rose native to the Isparta region of Turkey. The production of this variety for industrial purposes in Turkey (Lakes Region) commenced in 1888. At present, the only part of the *Rosa damascena* Mill. plant that is used is the flower. The flowers, flower stems, flower covers, and reproductive organs are used to obtain rose oil in Turkey without distinction. *Rosa damascena* Mill is not only an economically advantageous plant, but it also occupies a significant position among medicinal plants [18, 19]. Rose pomace is the byproduct of the distillation of rose, which is released into the environment as waste. Rose pulp and rose water have been demonstrated to possess antioxidant and antibacterial properties [11, 12]. It has been demonstrated that rose oil possesses a substantial phenolic substance content, with pulp water exhibiting a higher concentration of phenolic compounds than rose water. Flavonols are compounds that have exceptionally high antioxidant properties. Rose pulp contains a quantity of phenolic compounds nearly equivalent to that found in rose oil, and this concentration is significantly higher than that observed in rose water [22]. Acne, which can manifest at any age, is a condition that causes distress for the individual due to its tendency to attract attention. The development of acne is attributed to hormonal and nutritional factors. An increase in body fat is a contributing factor to the occurrence of acne [23]. The primary cosmetic benefits of facial masks can be attributed to their ability to provide rapid and profound moisturization, facilitate skin renewal and restoration, promote sebum absorption and elimination, and impart a rejuvenated appearance. Face masks are products that can be readily and uniformly distributed to the intended application area with the fingers. Once applied, the mask should be left in place and not allowed to flow [24]. From a physicochemical perspective, facial masks can be classified into three primary formulation types: emulsions, gels, and suspensions. Various formulations can be created with each of these, but facial masks are typically produced as simple combinations of a few fundamental ingredients [25]. As in our study, rose mask formulations include a variety of ingredients, including rose pulp, thermal water, thermal clay,

glycerin, aloe vera, hyaluronic acid, preservatives, EDTA, or sodium gluconate [26, 27]. The face mask prepared in our study was found to be effective in achieving its intended purpose.

4. CONCLUSION

The incorporation of rose pulp into paste-type acne mask formulations did not result in any alterations to the structural composition of the paste components. The properties of the paste were preserved for spreadability and sensation. Tests for the optimal formulation designated GP-AMF-10 and GP-AMF-8 demonstrated that rose pulp waste can be utilized safely in the production of masks.

5. MATERIAL and METHOD

In the study, rose pulp (Local Producer, Turkey), thermal water (Kızılay, Turkey), thermal clay (Sabunaria, Turkey), hyaluronic acid (Nature Pharmaceuticals, Turkey), glycerin (Galenik, Turkey), and Ethylhexylglycerin (Ashland) were used. The devices used were Milwaukee MW150 max (Szeged-Hungary), Rotational Viscometer PCE-RVI 10 (Meschede-Germany), Elektromag M5040 PS (Çerkezköy – Turkey). The sample preparation process was carried out according to the EPA 3015 method. It involved using the Milestone brand ETHOS ONE model microwave sample preparation unit and adding 5 mL of HNO_3 and 1 mL of H_2O_2 to 1 g of sample. The wet digestion method was used for this process. The pour plate method was used for microbiological analysis of the sample.

5.1. Formulation Study

The rose pulp is subjected to a low-temperature drying process, conducted in a manner that avoids exposure to solar radiation, in order to prevent the volatilisation of the phenolic compounds it contains. Sterile clay is then incorporated into the rose pulp and mixed thoroughly. Subsequently, hyaluronic acid and aloe vera are added to the thermal water, which is then fully dispersed under a mechanical mixer. The weight of the rose pulp and other solid ingredients (talc, kaolin, thermal clay, hyaluronic acid, EDTA) is determined with precision, from the lowest to the highest weight, and the ingredients are mixed. Subsequently, the requisite quantities of the liquid constituents (hyaluronic acid and aloe vera dispersion prepared with thermal water, glycerin, propylene glycol, and preservative) are measured and added to the solid materials in a manner that adheres to the geometric dilution principle, thereby forming a colloidal dispersion. The use of a mechanical mixer ensures the uniform dispersion of the ingredients.

Table 7. Composition of acne formulations containing Rose Pulp

Formulation	RP	TC %	T %	K %	G %	PG %	AV %	HA %	ETG %	PHE %	EDTA %	TW %
GP-AMF-1	0.5	35	2.5	2.5	1	1	0.5	0.2	0.1	0.5	0.1	56.2
GP-AMF-2	0.5	35	0	0	1	1	0.5	0.2	0.1	0.5	0.1	61.2
GP-AMF-3	0.5	35	5	0	1	1	0.5	0.2	0.1	0.5	0.1	56.2
GP-AMF-4	0.5	35	0	5	1	1	0.5	0.2	0.1	0.5	0.1	56.2
GP-AMF-5	0.5	35	5	5	1	1	0.5	0.2	0.1	0.5	0.1	51.2
GP-AMF-6	0.5	35	7.5	0	1	1	0.5	0.2	0.1	0.5	0.1	53.7
GP-AMF-7	0.5	35	0	7.5	1	1	0.5	0.2	0.1	0.5	0.1	53.7
GP-AMF-8	0.5	35	7.5	7.5	1	1	0.5	0.2	0.1	0.5	0.1	46.2
GP-AMF-9	0.5	25	7.5	7.5	1	1	0.5	0.2	0.1	0.5	0.1	56.2
GP-AMF-10	0.5	45	7.5	7.5	1	1	0.5	0.2	0.1	0.5	0.1	36.2

RP: Rose Pulp; TC: Thermal Clay; T: Talck; K: Kaolin; G: Glycerin; PG: Propylene Glycol; AV: Aloe Vera; HA: Hyaluronic Acid; ETG: Ethylhexylglycerin; PHE: Phenoxyethanol; EDTA: Ethylenediamine tetraacetic acid; TW: Thermal Water.

5.2 Physicochemical Controls

5.2.1. pH Measurement

The pH of cosmetic products applied to the skin should be compatible with the skin and should not cause irritation. The pH of an appropriate acne mask formulation is expected to be slightly acidic. The pH measurements of each prepared formulation were carried out in triplicate using a digital pH meter in accordance with the relevant cosmetic regulations.

5.2.2. Spreadability Assessment:

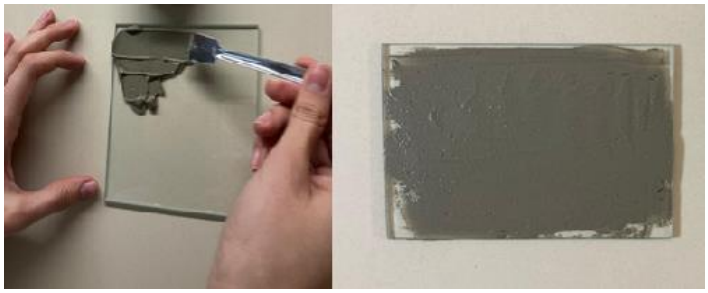

Figure 3. Spreadability evaluation on glass plaque

Rinse-off mask formulations should be straightforward to apply and remove from the skin. In terms of rheological properties, the formulation should remain in the application area and should not flow. To evaluate the applicability, a sensory evaluation was conducted to assess the ease of application, the sensory properties, the visual appearance, the ability to remain on the surface without flowing, and the user-friendliness of the mask. In order to assess the spreadability of the formulations, they were applied to a smooth, transparent glass surface, with minor modifications to the methodology employed by Beringhs et al. [19].

5.2.3. Drying time determination:

A drying time test is conducted to ascertain the time required for the formulations to dry fully following application to the skin. In order to ensure the reliability of the measurement results, the glass plate was measured at a total of 12 points with three measurements taken on each edge using callipers. The measurements yielded a glass plate thickness of 3.9 mm. Approximately 2.0 g of each formulation was distributed onto a 12×12 cm glass plate to create a uniform mask layer with a thickness of approximately 0.2 mm on the 3.9 mm thick glass plate. The glass plate was placed in an oven maintained at a temperature of 37.0 ± 2.0°C, which was deemed to be representative of the temperature of the skin. The surface of the mask applied to the glass plate was observed until it was deemed to be completely dry. The results are presented as the mean of three measurements [19].

Figure 4. Application of 0.2 mm thick mask on glass plate and determination of drying time (37 °C-oven, n=3)

5.2.4. Film Making Performance:

It is anticipated that rinseable mask formulations will be capable of forming a film on the skin, spreading in a homogeneous manner and completely covering the area to which they are applied. Approximately 2.0 g of each formulation was applied to a 12×12 cm glass plate to form a uniform mask layer with a thickness of approximately 0.2 mm, which was then dried on a 3.9 mm thick glass plate. The glass plate was maintained in an oven maintained at a temperature of 37.0 ± 2.0°C, which is representative of the temperature of the skin, until the completion of the drying process. Following the drying time of the mask formulations, an examination of the surface covered with film was conducted using a microscope. This

examination was conducted to ascertain whether film formation had occurred and whether the film was homogeneous [19].

5.3. Rheological Studies

The apparent viscosity was determined using a rotational viscometer (PCE Instruments, Hamburg, Germany), calibrated before use. A rheometer is a device used to measure viscosity, calculating the torque required to rotate the spindles within the fluid. The torque is related to the viscosity of the fluid. In this experiment, samples were measured with an L2 spindle at 6 rpm for 120 seconds. The slip rate is calculated using the dimensions of the shaft, the rotation speed, and the gap between the shaft and the container. We set the gap to 1.25 mm, which gave us a ratio of 1.2 between the container diameter and the shaft. We calculated the sliding speed using the formula (1).

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

In the equation, "y" represents the sliding speed, "Ni" denotes the rotation speed, and "R0" and "Ri" are the radii of the cup and shaft. The apparent viscosity values were plotted as a function of shear rate and fitted to the Ostwald-de Waele equation (Eq. x) [20].

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

K is the viscosity coefficient and n is the flow behavior index calculated from exponential regression [21].

5.4. Calculation of The İnterval of Safety (MoS)

The rose pulp acne mask formulation has a systemic exposure dose of 1.54 g/day. This is based on the product being used once a day on a 565 cm² area of skin. The safety margin values are based on formula 4.

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

5.5. Stability Study

Stability studies were conducted to ascertain whether any changes in appearance, color, pH, viscosity, or microbiological growth occurred over six months when the product was stored in a refrigerator, room, and oven (45°C).

5.6. Microbiological Analysis

In microbiological analysis, pathogens that should not be present in 1 gr or ml of cosmetic product according to ISO 17516, 2014 Standard; It has been shown in studies on E. coli (ISO 21150), S. aureus, P. Aeruginosa (ISO 22717) and C. albicans (ISO 16212) [20]. The microbiological test formulation utilized in the present study was disinfected with 70% ethanol. A solution of 5 g/L polysorbate 80 and 90 ml sodium chloride-buffered peptone (TSP) was prepared to dissolve the sample. Subsequently, 10 g of the sample was added and allowed to dissolve in a water bath for approximately 10–15 minutes. A volume of 1 mL of the diluted sample was poured into a sterile empty petri plate using a pipette. Approximately 15 mL of warm agar medium (~45-50^{o}C) was prepared by pouring it onto the sample in the petri dish. The sample and liquid agar were mixed gently. The agar was allowed to solidify at room temperature. The optimal incubation period was maintained for each microorganism at the optimal temperature. In the case of reproduction, colonies that are visible to the naked eye are counted using the following formüla (4).

CFU/ml = Total number of colonies obtained x dilution factor/ Sample volüme (4)

Acknowledgments: Not applicable

Author Contributions: Concept – A.k., Design – A.K., B.I., Source – B.I., Materials – A.K., Data Collection and/or Processing – A.K., Analysis and/or Interpretation – A.K., B.I., Literature Review – B.I., Writing – B.I., Critical Reviews - A.K., B.I.,

Conflicts of Interest: The authors declare that they have no financial interest.

Data Availability: Not applicable.

REFERENCES

[1] Kurokawa I, Nakase K. Recent advances in understanding and managing acne. F1000Res. 2020 Jul 29;9:F1000 Faculty Rev-792. https://doi.org/10.12688/f1000research.25588.

[2] Liao JC, Deng JS, Chiu CS, Huang SS, Hou WC, Lin WC, Huang GJ. Chemical compositions, anti-inflammatory, antiproliferative and radical-scavenging activities of *Actinidia callosa* var. ephippioides. Am J Chin Med. 2012;40(5):1047-1062. https://doi.org/10.1142/S0192415X12500772.

[3] Schachner LA, Alexis AF, Andriessen A, Berson D, Gold M, Goldberg DJ, Hu S, Keri J, Kircik L, Woolery-Lloyd H. Insights into acne and the skin barrier: Optimizing treatment regimens with ceramide-containing skincare. J Cosmet Dermatol. 2023;22(11):2902-2909. https://doi.org/10.1111/jocd.15946.

[4] Yazici EK, Eryilmaz M. Investigation of the microbiological contamination of used lipsticks and mascaras. J Fac Pharm Ankara. 2023; 47: 269–276. https://doi.org/10.33483/jfpau.1209451

[5] Koç DE, Avcı M. Climate change impacts on the potential distribution of *Taxus baccata* L. in the Eastern Mediterranean and the Bolkar Mountains (Turkey) from Last Glacial Maximum to the future. Eurasian J Forest Sci. 2018; 6(3): 69 – 82. https://doi.org/10.31195/ejejfs.435962

[6] Wang F, Xiang L, Sze-Yin Leung K, Elsner M, Zhang Y, Guo Y, Pan B, Sun H, An T, Ying G, Brooks BW, Hou D, Helbling DE, Sun J, Qiu H, Vogel TM, Zhang W, Gao Y, Simpson MJ, Luo Y, Chang SX, Su G, Wong BM, Fu TM, Zhu D, Jobst KJ, Ge C, Coulon F, Harindintwali JD, Zeng X, Wang H, Fu Y, Wei Z, Lohmann R, Chen C, Song Y, Sanchez-Cid C, Wang Y, El-Naggar A, Yao Y, Huang Y, Cheuk-Fung Law J, Gu C, Shen H, Gao Y, Qin C, Li H, Zhang T, Corcoll N, Liu M, Alessi DS, Li H, Brandt KK, Pico Y, Gu C, Guo J, Su J, Corvini P, Ye M, Rocha-Santos T, He H, Yang Y, Tong M, Zhang W, Suanon F, Brahushi F, Wang Z, Hashsham SA, Virta M, Yuan Q, Jiang G, Tremblay LA, Bu Q, Wu J, Peijnenburg W, Topp E, Cao X, Jiang X, Zheng M, Zhang T, Luo Y, Zhu L, Li X, Barceló D, Chen J, Xing B, Amelung W, Cai Z, Naidu R, Shen Q, Pawliszyn J, Zhu YG, Schaeffer A, Rillig MC, Wu F, Yu G, Tiedje JM. Emerging contaminants: A One Health perspective. Innovation (Camb). 2024;5(4):100612. https://doi.org/10.1016/j.xinn.2024.100612.

[7] Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021;9(10):2041. https://doi.org/10.3390/microorganisms9102041

[8] Chaachoouay N, Zidane L. Plant-derived natural products: A source for drug discovery and development. Drugs Drug Candidates. 2024;3(1): 184-207. https://doi.org/10.3390/ddc3010011

[9] Nematian MA, Bahramynia B, Baghaeifar Z. Chemical composition of the essential oils isolated from *Phlomis olivieri* Benth (Lamiaceae) in four western provinces in Iran. Int J Second Met. 2024,11: 2, 315–332.

[10] Akram M, Riaz 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. J Pharm Pharmacol. 2020;72(2):161-174. https://doi.org/10.1111/jphp.13185.

[11] Baydar H, Göktürk Baydar N. Yağ Gülü (Rosa damascena Mill.)'nde Distilasyon Ürünlerinin Uçucu Yağ ve Fenolik Madde İçerikleri ile Antiradikal ve Antioksidan Aktiviteleri. J Agric Sci. 2017;23:1-9.

[12] Androutsopoulou C, Christopoulou SD, Hahalis P, Kotsalou C, Lamari FN, Vantarakis A. Evaluation of essential oils and extracts of rose geranium and rose petals as natural preservatives in terms of toxicity, antimicrobial, and antiviral activity. Pathogens 2021; 10. https://doi.org/10.3390/pathogens10040494.

[13] Ersan R, Başayiğit L. Ecological modelling of potential Isparta Rosa areas (Rosa damascena Mill.). Ind Crops Products 2022; 176: 114427. http://dx.doi.org/10.1016/j.indcrop.2021.114427.

[14] Kurt AA, Aslan I, Duman G. Next-generation natural baby barrier cream formulations: Physicochemical analysis and safety assessment. J Cosmet Sci. 2021; 72(2):173-188.

[15] Głaz P, Rosińska A, Woźniak S, Boguszewska-Czubara A, Biernasiuk A, Matosiuk D. Effect of commonly used cosmetic preservatives on healthy human skin. Cells 2023; 3:12(7):1076. https://doi.org/10.1016/j.indcrop.2021.114427.

[16] Moser CL, Meyer BK. Comparison of compendial antimicrobial effectiveness tests: A review. AAPS PharmSciTech 2011; 12: 222–226. https://doi.org/10.1208/s12249-010-9575-9.

[17] Özçelik H, Muca B, Özavci A.M. Isparta Yağgülü (Rosa x damascena Mill.) Yağı ve Çiçeklerinin Strese Bağlı Nörolojik ve Psikiyatrik Hastalıklara Etkileri. Biyoloji Bilimleri Araştırma Dergisi. 2011; 4 (2): 99-105.

[18] Yuca H, Tekman E, Civaş A, Karakaya S, Öztürk G, Demirci B, Karaca A, Güvenalp Z. Comparison of essential oils and secretion structures of *Rosa damascena* Mill. grown in Iğdır and Isparta (Turkey). Sakarya University J Sci. 2023; 27: 523–529. https://doi.org/10.16984/saufenbilder.1197825.

[19] Alizadeh Z, Fattahi M. Essential oil, total phenolic, flavonoids, anthocyanins, carotenoids and antioxidant activity of cultivated Damask Rose (*Rosa damascena*) from Iran: With chemotyping approach concerning morphology and composition. Sci Hortic. 2021; 288. https://doi.org/10.1016/j.scienta.2021.110341.

[20] E. Lasseuguette, D. Roux, ve Y. Nishiyama, "Rheological properties of microfibrillar suspension of TEMPO-oxidized pulp", Cellulose, c. 15, sy 3, ss. 425-433, Haz. 2008, doi: 10.1007/s10570-007-9184-2

[21] P. J. do Amaral Sobral vd., "Rheological and Viscoelastic Properties of Chitosan Solutions Prepared with Different Chitosan or Acetic Acid Concentrations", Foods, c. 11, sy 17, Art. sy 17, Oca. 2022, doi: 10.3390/foods11172692.

[22] Ryguła I, Pikiewicz W, Kaminiów K. Impact of diet and nutrition in patients with acne vulgaris. Nutrients 2024; 16(10): 1476. https://doi.org/10.3390/nu16101476.

[23] Nilforoushzadeh MA, Amirkhani MA, Zarrintaj P, Salehi Moghaddam A, Mehrabi T, Alavi S, Mollapour Sisakht M. Skin care and rejuvenation by cosmeceutical facial mask. J Cosmet Dermatol. 2018;17(5):693-702. https://doi.org/10.1111/jocd.12730.

[24] Jadach B, Mielcarek Z, Osmałek T. Use of Collagen in Cosmetic Products. Curr Issues Mol Biol. 2024;46(3):2043-2070. https://doi.org/10.3390/cimb46030132.

[25] Fitri R, Syahputra HD, Nasri N, Kaban VE, Rani Z. Formulatıon of A biocellulose mask contaınıng the essence of *Aloe vera* (L.) Burm.F combınatıon wıth vıtamın E as antı-agıng. ScienceRise: Pharm Sci. 2024; 6(40):21 https://doi.org/10.15587/2519-4852.2022.268987.

[26] O'Reilly Beringhs A, Rosa JM, Stulzer HK, Budal RM, Sonaglio D. Green clay and aloe vera peel-off facial masks: response surface methodology applied to the formulation design. AAPS PharmSciTech. 2013;14(1):445-455. https://doi.org/10.1208%2Fs12249-013-9930-8.

[27] Kurt AA, Ibrahim B. Development and microbiological evaluation of natural diaper rash (Diaper Dermatitis) cream formulations. J Immunol Clin Microbiol. 2024; 9(1): 1-11. https://doi.org/10.58854/jicm.140277.