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물오리나무 엑스의 폴리에틸렌글리콜 연고제 설계 및 가속 안정성 평가

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Polyethylene Glycol-based Ointment Formulations of *Alnus Sibirica* Extract and Their Accelerated Stability Assessments

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초록: 천연물 유래 면역 조절제인 물오리나무 엑스는 불안정성으로 인해 연고제 개발에 제한적이었다. 본 연구에서는 폴리에틸렌글리콜을 기반으로 한 안정화된 물오리나무 엑스 연고를 개발하였다. 허쉘-버클리 모델에 대입하였을 때 유동 거동 지수는 0.41~0.97로 계산되었으며, 전단박화 유동을 확인하였다. 사람을 대상으로 한 관능 평가에서 폴리에틸렌글리콜 연고가 습윤성 및 수세성 측면에서 백색바셀린 연고보다 높은 점수를 나타내었다. 물오리나무 엑스의 온도의존성 분해반응은 1차 속도식을 따랐으며, 40 °C 가속 안정성 시험에서 폴리에틸렌글리콜 연고(P3)의 유효기간이 527일로 가장 길었다. 또한 계산된 2.17의 Q_{10} 값을 이용하였을 때, P3의 유효기간은 25 °C에서 4년을 초과하는 것으로 추정되었다. 따라서 우수한 안정성을 갖는 제품 개발에 P3 제제가 적절한 후보군이라 할 수 있다.

Abstract: Ointment formulation development of *Alnus sibirica* extract (ASEx), a natural immunomodulator, has been limited because of instability problem. In this study, stabilized ASEx-containing ointments were developed using polyethylene glycol (PEG) base. By the Herschel–Bulkley model, the flow behavior indices were calculated as 0.41~0.97, indicating a shear-thinning flow. The PEG ointments were superior to other comparative formulation, white petrolatum-based ointment, in terms of moistness and removal capacity in a simplified sensory assessment by human volunteers. ASEx degradation followed first-order kinetics with temperature dependence. From accelerated stability assessments, a selective formulation (P3) was found to have longest shelf life of 527 days at 40 °C. Furthermore, by applying a Q_{10} value of 2.17, the shelf life of P3 at 25 °C was estimated to exceed four years. Thus, we conclude that the P3 formulation may be an appropriate candidate for development of a commercial product with good long-term stability.

Keywords: *Alnus sibirica* extract, polyethylene glycol, ointments, stability, zinc stearate.

Introduction

Alnus sibirica (AS) is a tree of the family Betulaceae that is found in damp areas of mountain valleys and has been used in Oriental traditional medicines as a remedy for fever, hemorrhages, burn injuries, diarrhea, and alcoholism.[1] The extract of AS (ASEx) contains various phenolic compounds such as flavonoids, tannins, and triterpenoids, including a series of diarylheptanoids.[2-4] These compounds have antioxidant and anti-inflammatory properties. In particular, oregonin (ORG) and hirsutenone (HST) have been isolated as biologically active diarylheptanoids.[5] ORG is a glucopyranoside of HST (aglycon form; 1,7-*bis*-(3,4-dihydroxyphenyl)-5-hydroxyheptane-3-on); thus, ORG is water-soluble, while HST is water-insoluble. Previous studies have reported that ORG suppressed inflammation in rabbit macrophages by regulating NF-kB signaling,[6] reduced cellular lipid accumulation and pro-inflammatory cytokine secretion in primary human macrophages,[7] and inhib-

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ited lipopolysaccharide-induced nitric oxide synthase gene transcription in macrophages and microglia.[8] Taking advantage of these effects, ORG and HST have been investigated as natural immunomodulators to treat autoimmune diseases, including atopic dermatitis.[9,10]

Atopic dermatitis is a chronic, incurable immune disease that occurs due to a combination of genetic and environmental factors and involves an increased concentration of cytokines secreted by Th2 cells that activate the host's allergic response, ultimately upregulating B cell synthesis of Immunoglobulin E.[11] In addition, inflammation-related enzymes such as inducible nitric oxide synthase, cyclooxygenase-2, and 5-lipoxygenase are important factors that promote inflammatory responses in dermatitis.[12] Topical application of corticosteroids along with frequent emollient use has been the mainstay of treatment for atopic dermatitis. Although corticosteroids are highly effective, their associated local and systemic side effects such as skin thinning, atrophic changes including telangiectasia, purpura, striae, and adrenal gland suppression limit their long-term use.[13] Alternatively, less toxic compounds including natural immunomodulators have received increased focus as possible treatment approaches.[14,15]

Various efforts to develop topical formulations for polyphenols such as ORG or HST have been attempted. Previously, we formulated HST-containing oleaginous ointments and creams[16] in which the stability of HST modestly increased by adding various antioxidants; however, these compounds remained unsatisfactory for practical development. Additionally, nanostructured lipid carrier formulations were investigated to stabilize HST in the presence of antioxidants.[17] A Tat peptide-admixed elastic liposomal system was formulated to improve the dermal delivery of HST.[12] ORG was formulated with various types of ointment vehicles, and the drug release characteristics were evaluated.[18] Topical cream and gel formulations containing polyphenols extracted from Echinacea purpurea were developed and evaluated for stability.[19] However, none of these formulations were able to attain a two-year shelf life for storage in ambient conditions, a prerequisite for commercial development. Because of this instability problem, the practical development of topical preparations containing these diarylheptanoids remains limited.

Therefore, in the present study, ASEx was formulated with two ointment base types: a polyethylene glycol (PEG) base, or white petrolatum-based ointments in the presence or absence of zinc stearate as a stabilizing agent. The physical characteristics of the formulations were evaluated in terms of viscosity and sensory tests. Accelerated stability assessments of the different ointments were conducted based on first-order kinetic analyses of ORG in ASEx. Applying the Q_{10} method, the shelf lives of ASEx-containing ointments were estimated.

Experimental

Materials. ASEx (14.9% ORG content) was supplied by Dongkook Pharm. Co., Ltd. (Seoul, Korea). ORG (purity > 92% by HPLC) was supplied by the Pharmacognosy laboratory at the College of Pharmacy in Chung-Ang University, Seoul, Republic of Korea. Beeswax, PEG 400, propylene glycol, sodium stearate, and zinc stearate were purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). PEG 4000 was purchased from Sanyo Chemical Industries, Ltd. (Kyoto, Japan). White petrolatum was purchased from Samchun Pure Chemical Co., Ltd. (Pyung-taek, Korea). All other chemicals and solvents purchased from commercial sources were of analytical reagent grade.

Preparation of ASEx-Containing Ointments. As listed in Table 1, PEG-based (P1–P3) and white petrolatum-based (V1 and V2) ointments containing ASEx were prepared using the reported melt fusion method.[20,21] Briefly, all constituents except for ASEx and zinc stearate were melted at 60 °C in a water bath (Sb-1300; Sunil Eyela Co., Seongnam, Korea) and thoroughly mixed; then, ASEx was added. For comparison, zinc stearate was added only for P3 and V2 ointments. The mixture was stirred at 60 °C with a polytetrafluoroethylene-coated magnetic bar until homogeneous. Then, the mixture was cooled to 25 °C under gentle stirring. Separately, the P3 formulation was further varied with different contents of zinc stearate or sodium stearate, with the final volume complemented with distilled water. All ointments were subjected for ageing at 25 °C for 24 h before use.

HPLC Determination of ORG in ASEx. ASEx was dissolved in distilled water (1 mg/mL) and assayed for ORG content using HPLC analysis. For the calibration curve, an ORG-dissolved stock solution (0.5 mg/mL) was prepared and serially diluted. Solution samples were filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane filter (Whatman International Ltd., Kent, UK); then, 20 μL of filtrate was injected into an Agilent Infinity 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a 1260 Quat pump VL, 1260 TCC (Column heater), and 1260 DAD WR (detector), and the data were collected using Agilent Empower Pro software. Using a C18 column (250×4.6 mm,

Table 1. Compositions of ASEx-Containing Ointments

Compositions (g)	PEG base			White petrolatum base	
	P1	P2	P3	V1	V2
ASEx	1.0	1.0	1.0	1.0	1.0
Polyethylene glycol 4000	23.0	23.0	23.0	-	-
Polyethylene glycol 400	62.0	62.0	62.0	-	-
Beeswax	-	-	-	5.0	5.0
Propylene glycol	-	-	-	15.0	15.0
Zinc stearate	-	-	5.0	-	5.0
White petrolatum	-	1.5	1.5	q.s. ad 100	q.s. ad 100
Water	q.s. ad 100	q.s. ad 100	q.s. ad 100	-	-

ASEx, *Alnus sibirica* extract.

5 μm; Shiseido, Tokyo, Japan), the mobile phase system consisted of A (0.1% phosphoric acid) and B (acetonitrile), with a varied gradient according to the following program: 10 min (95% A), 35 min (75% A), 40 min (55% A), 45 min (40% A), and 55 min (95% A). Analyses were performed at a flow rate of 1.0 mL/min at 25 ºC, and the column eluent was monitored at 280 nm. The intra- and inter-day precision and accuracy of the ORG assay in ASEx solution were determined in triplicate.

Assay Validation of ASEx in Ointments. Defined amounts of ASEx-containing ointments (P3 and V2) were accurately weighed and dissolved in a 10-fold volume of an extractant composed of methanol and distilled water (50:50, [v/v]). For complete extraction, the dissolved solution was heated at 60 ºC in a water bath and sonicated for 30 min at 40 ºC using a water bath-type sonicator (47 kHz; Branson 2210R-DTH; Branson Ultrasonics Corporation, Danbury, CT, USA). The solution was filtered through a 0.45 μm PVDF membrane filter and injected for the HPLC assay. The intra- and inter-day precision and accuracy of the ASEx assay for the different ointments were determined in triplicate.

Rheological Observation. Rheological assessments were carried out using the Advanced Rheometric Expansion System (ARES; Rheometric Scientific, U.K) equipped with parallel plates (25 mm in diameter) with a gap of 1.0 mm. Experiments were conducted under a steady shear flow at 25 ºC. Shear rates ranged from 0.1-100 s^{-1}. Separately, viscosity measurements for the P3 formulations with different contents of zinc stearate or sodium stearate were performed at 25 ºC using a rheometer (MCR 102; Anton Paar, Ostfildern, Germany) with a 25 mm parallel plate (PP25; Anton Paar, Ostfildern, Germany) at a shear rate of 1.0 s^{-1}. All experiments were performed in triplicate and averaged.

Sensory Evaluation in Human Volunteers. Informed consent was received from all subjects before participation in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Chung-Ang University (Protocol number: ID-1041078-201912-HRBM-366-01). Twelve healthy volunteers aged 25-55 living in and around Seoul were chosen for the panel and were familiarized with the terms, evaluation procedures, and rating scales. The panel members were educated on the general concept of the study through a detailed explanation of the test, such as descriptions of the scales for the evaluation parameters and reference points, during tutorial sessions. Subsequently, panelists evaluated the various ointment samples according to the samples' sensory attributes: appearance, moistness, spreadability, odor, and capacity for removal, as shown in Table 2. For each assessment, the panelists evaluated five ointment samples presented in the same containers labeled with three-digit code numbers. This assessment was carried out in triplicate, and the values were averaged.

Aqueous Stability of ASEx at Different Temperatures. Aliquots of ASEx dissolved in aqueous solution (1 mg/mL) were transferred to 10 mL Teflon-capped vials and sealed with parafilm to shut off the evaporation of water; then, the samples were stored at different temperature of 25, 40, and 50 ºC for 10 days. At predetermined time points, 300 μL of sample solution were removed from the vial and filtered through a 0.45 μm PVDF membrane filter, and the filtrates were subjected to HPLC analysis as described above. The first-order degradation rate constants were calculated from the semi-logarithmic plot of the amount of remaining ORG in ASEx against time, and degradation's temperature dependence was analyzed by the Arrhenius plot.

Table 2. Sensory Attributes and Descriptive Terms for Simplified Sensory Evaluations

Attributes	Definition	Descriptive terms (score)
Appearance	The degree to which the product gives a feeling of homogeneity.	poor (1), acceptable (2), fair (3), good (4), optimal (5)
Moistness	The degree to which the product moisturizes the skin.	poor (1), acceptable (2), fair (3), good (4), optimal (5)
Spreadability	The degree to which the product spreads easily.	poor (1), acceptable (2), fair (3), good (4), optimal (5)
Odor	The degree to which the product gives a smell.	grossness (1), unpleasant (2), normal (3), pleasant (4), very pleasant (5)
Capacity for removal	The degree to which the product washes off the skin using water.	unwashable (1), hardly washable (2), washable (3), easily washable (4), very easily washable (5)

Accelerated Stability Tests of ASEx-Containing Ointments. For the accelerated stability test, ASEx-containing ointments were kept in glass vials and stored at 40 ºC and 75% relative humidity using the Climacell 707 display cabinet (MMM Medcenter Einrichtungen, Munich, Germany). At predetermined time points (0, 30, 90, and 120 day), ORG was extracted from the ointments as described above. The extract was filtered through a 0.45 μm PVDF membrane filter, and an aliquot (20 μL) of filtrate was introduced into HPLC. Separately, changes in apparent consistency were examined for , all ointments that were kept in 50 mL Falcon tubes and stored in the same conditions for 120 days.

Results and Discussion

Characterization of ASEx-Containing Ointments. ASEx-containing ointments were successfully prepared. PEG-based ointments (P1, P2, and P3) and white petrolatum-based ointments (V1 and V2) were prepared as hydrophilic and oleaginous types, respectively. For the PEG-based ointments, PEG 400 and PEG 4000 were used as the main constituents (P1), and a small amount of white petrolatum and/or zinc stearate was added (P2 and P3) as a viscosity modifier and stabilizer. For the white petrolatum-based ointments, V1 was composed of white petrolatum, propylene glycol, and beeswax, while V2 further contained a small amount of zinc stearate. Throughout all ointment formulations, content uniformity was maintained in the range of 100 ± 5% ASEx content.

Flow curves of the different ASEx-containing ointments are shown in Figure 1. All ointments demonstrated non-Newtonian flow behavior. To further analyze the rheological behavior of the ointments, the Herschel–Bulkley model was employed with the following eq. (1):[22]

$$\tau = \tau_0 + \kappa\gamma^n \quad (1)$$

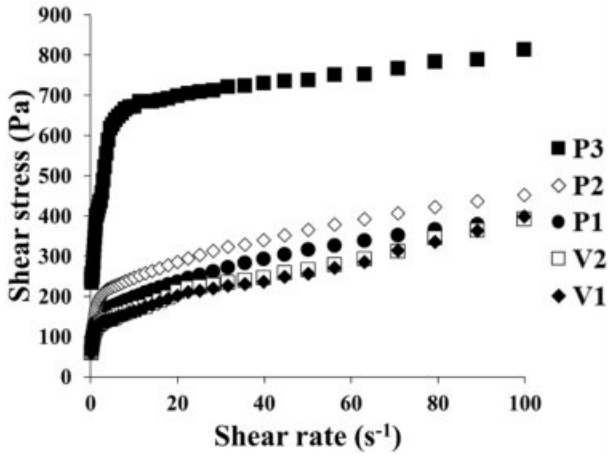

Figure 1. Flow curves of ASEx-containing ointments.

where τ is shear stress, τ_0 is yield stress, γ is shear rate, κ is the consistency index, and n is the flow behavior index. The flow curves fit well with good correlation coefficients ($R^2 > 0.95$), and the yield stress was determined from the intercept of the linear correlation between the first three or four plot points.[23] The values for these rheological parameters are listed in Table 3. P3 showed a much greater τ_0 value compared to the other ointments; the τ_0 values for the ointments are ranked P3 > P2 ≥ V2 > P1 > V1. The consistency index (κ) represents the viscous nature of the semi-solid formulations[24] and was greatest in the P3 formulation. The flow behavior index (n) indicates the degree of shear sensitivity, and lower n values indicate greater viscosity reliance on the shear rate.[25] Flow behavior with $n = 1$ corresponds to Newtonian flow, $n < 1$ indicates a shear-thinning fluid, while $n > 1$ indicates a dilatant fluid.[26] All ointments demonstrated values in the range of 0.26~0.97, indicating shear-thinning flow behavior.

Sensory Evaluation of ASEx-Containing Ointments. In recent years, several techniques have been developed to objectively evaluate skin properties in dermato-cosmetic research.[27]

Table 3. Characteristics of ASEx-Containing Ointments

Characteristics	P1	P2	P3	V1	V2
ASEx content (w/w, %)	100.67 ± 0.67	101.34 ± 2.01	97.99 ± 2.68	97.32 ± 1.34	99.33 ± 2.68
τ_0 (Pa)[a]	131.17 ± 1.19	168.58 ± 0.82	472.78 ±13.82	79.30 ± 3.32	127.27 ± 4.41
n^a	0.63 ± 0.02	0.57 ± 0.01	0.26 ± 0.01	0.97 ± 0.03	0.82 ± 0.02
κ (Pa·s^n)[a]	14.96 ± 2.27	20.85 ± 0.62	87.39 ± 14.49	2.97 ± 0.07	5.97 ± 0.52

[a]Calculated by the Hershel–Bulkley equation: τ_0, yield stress; n, flow behavior index; κ, consistency index. Data are presented as the mean ± SD (n=3). ASEx, *Alnus sibirica* extract.

Table 4. Sensory Assessment Results for Various Ointments

Attributes	P1	P2	P3	V1	V2
Appearance	3.17 ± 0.39	3.25 ± 0.87	3.42 ± 0.67	3.25 ± 0.87	3.42 ± 0.79
Moistness	2.83 ± 0.94	2.83 ± 0.94	3.33 ± 0.89	2.42 ± 0.79	2.50 ± 0.90
Spreadability	3.25 ± 0.45	3.33 ± 0.49	2.83 ± 0.72	3.33 ± 0.65	3.25 ± 0.87
Odor	3.08 ± 0.51	3.00 ± 0.60	3.08 ± 0.51	3.00 ± 0.60	3.08 ± 0.67
Capacity for removal	3.58 ± 0.90	3.33 ± 0.98	3.17 ± 0.83	1.83 ± 0.72	1.75 ± 0.62

Data are presented as the mean ± SD (n=3).

Quantitative descriptive analyses of cosmetic products have been widely used to evaluate the sensory characteristics of cosmetics and cosmetic ingredients.[28] However, given the diversity of sensory attributes, it would be too comprehensive, time-consuming, lengthy, and expensive to conduct such analyses.[29] Thus, in this study, a simplified sensory assessment was performed by employing the sensory attributes of appearance, moistness, spreadability, odor, and capacity for removal.

Twelve panelists were recruited and instructed to grade their feelings on a score of 1 (poor) to 5 (optimal). These results are listed in Table 4. Despite the limited number of panel members, several significant results were found. For the appearance attribute, none of the panelists gave any of the compounds a score of 1, and the average scores ranged from 3.17–3.42, indicating a fair to good impression. For the moistness attribute, the PEG-based ointments were superior to the white petrolatum-based ointments. Two panelists gave a score of 5 (optimal) for P3, while two panelists gave a score of 1 (poor) to V1 and V2. Although both PEG and white petrolatum have been reported as good moisturizing agents,[30,31] the white petrolatum-based ointments showed lower moistness scores in this experiment. This might be attributed to individual panel members' variations in sensation. For the spreadability attribute, P3 showed the lowest score of 2.83 (acceptable to fair), while V2 was given a score of 5 (optimal) by one panelist. These results were considered to be closely related to rheological properties: P3 has a high viscosity and therefore yielded a lower score compared to other ointments with low viscosity. For the odor attribute, none of the panelists gave scores of 1 (grossness) or 5 (very pleasant), yielding a score of approximately 3 (normal) for all ointments. Small standard deviations were also observed, indicating little difference between the panelists' evaluations. Finally, regarding the capacity for removal by washing with water, PEG-based ointments showed a value of 3.17– 3.50 (washable to easily washable), which clearly surpassed the white petrolatum-based ointments that were graded with values of 1.75–1.83 (unwashable to hardly washable). Because PEG polymers are water-soluble and do not hydrolyze or support mold growth, PEG makes a good base for washable ointments and can be formulated to have a soft-to-hard consistency.[32] In contrast, white petrolatum-based ointments are mainly composed of oleaginous ingredients, consequently making them hard to remove by washing with water alone.

HPLC Assay Validation of ORG in ASEx. A typical chromatogram of an ASEx-dissolved stock solution is shown in Figure 2 (chromatogram a). ORG was separated with an acceptable peak resolution at a retention time of 23.40 min, while the trace amount of HST was identified with a noise peak at a retention time of 35.30 min. Upon integration, the peak area of ORG was 11-fold greater than that of HST (data not shown); thus, ORG was selected as a marker compound for the stability-indicating assay in the quality control analyses. This peak pattern was identical in both the P3 ointment (chro-

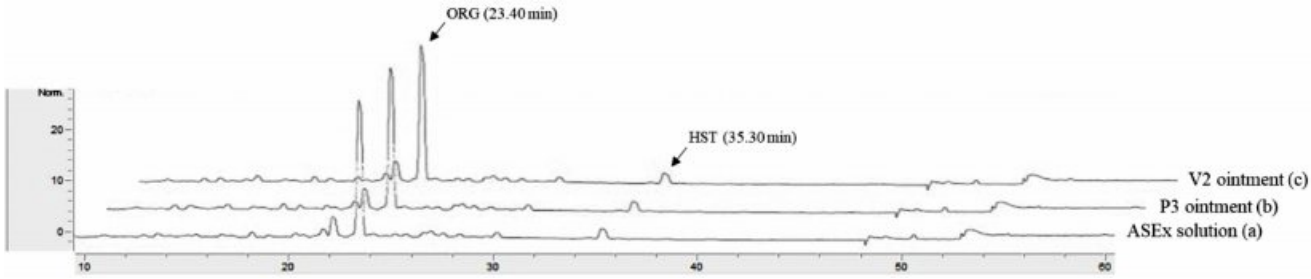

Figure 2. Typical HPLC chromatograms of ASEx in a stock solution (a); P3 ointment (b); V2 ointment (c).

Table 5. Intra- and Inter-day Validation of the ORG Assay in a Stock Solution and Representative Ointments of P3 and V2

Spiked concentration (μg/mL)	P3			V2		
	Detected concentration (μg/mL)	Precision (RSD)[a]	Accuracy (% DEV)[b]	Detected concentration (μg/mL)	Precision (RSD)[a]	Accuracy (% DEV)[b]
Intra-day validation						
25	24.73 ± 1.46	5.34	1.69	24.62 ± 1.75	5.36	0.04
50	48.75 ± 1.47	3.60	2.38	48.67 ± 1.67	3.11	2.58
100	97.60 ± 4.03	3.55	2.84	98.07 ± 2.69	5.98	-0.68
200	200.36 ± 5.31	1.80	1.81	201.03 ± 3.88	2.25	-1.35
300	302.21 ± 5.90	1.81	-0.94	302.74 ± 2.83	1.75	-0.46
Inter-day validation						
25	24.84 ± 1.73	6.97	0.62	24.85 ± 1.51	6.07	0.59
50	48.75 ± 1.20	2.45	2.50	48.53 ± 1.60	3.30	2.93
100	97.77 ± 4.88	4.99	2.23	98.77 ± 5.97	6.04	1.23
200	199.75 ± 3.98	1.99	0.13	202.29 ± 4.32	2.13	-1.15
300	302.53 ± 1.86	0.61	-0.84	301.12 ± 5.35	1.78	-0.37

[a]Calculated by SD×100/mean. [b]Calculated by (spiked concentration – measured concentration)×100/spiked concentration. Data are presented as the mean ± SD (n=3). RSD, Relative standard deviation; % DEV, percentage deviation.

matogram b) and the V2 ointment (chromatogram c) as representative PEG-based and white petrolatum-based ointments, respectively, indicating no interference of the ointment base on the ORG assay. The calibration curve of the peak area ratio of ORG *versus* ORG concentration resulted in a regression equation of $y = 10252x - 16234$ ($R^2 = 0.9998$) in the range of 1–400 μg/mL. Intra- and inter-day validations for accuracy and precision analysis for the ASEx solutions and ointments were established. For ASEx solutions in the range of 25–300 μg/mL, the intra- and inter-day accuracies ranged from -1.7 to 4% and from -0.6 to 3.6%, respectively. Intra- and inter-day precisions ranged from 2.1 to 6.8% and from 1.3 to 5.4%, respectively. ORG assays for both the P3 and V2 ointments were also validated (Table 5). For both ointments, the intra- and inter-day validation data were mostly within a 6% deviation. All validation data were acceptable for both ointments, as the criteria for accuracy were within the 15% deviation, and precision was within a 15% relative standard deviation from the normal values.[33]

Degradation Kinetics of ORG in the ASEx Solution. As shown in Figure 3(A), the semi-log plot of the remaining ORG *versus* time gave a straight line with good linearity ($R^2 > 0.9$), indicating the first-order degradation of ORG following eq. (2):

$$C/C_0 = e^{-kt} \quad (2)$$

where C/C_0 is the fraction of remaining ORG in the aqueous solution at the predetermined time point t, and k is the rate constant (day^{-1}). Using the regression function of Microsoft Excel

2019, k values ($\times 10^{-2}$) were obtained as 1.35 (25 °C), 3.39 (40 °C), and 7.35 (50 °C). ORG was unstable in aqueous solution, with a short half-life of 51.3 days even at 25 °C. The degradation of ORG was accelerated as temperature increased. The temperature dependence of ORG degradation was further analyzed using the following Arrhenius eq. (3):

$$k = A \cdot e^{-Ea/RT} \quad (3)$$

where E_a is the activation energy, A is the frequency factor, T is the absolute temperature, and R is the gas constant. As shown in Figure 3(B), the semi-log plot of the rate constants versus reciprocal absolute temperature showed a linear relationship with a good correlation ($R^2 = 0.9925$). The activation energy of ORG, calculated from the slope of the regression line, was 53.60 kJ/mole, which is greater than that of HST (16.53 kJ/mole) as reported in the literature.[34] The higher the activation energy, the less likely that the degradation reaction

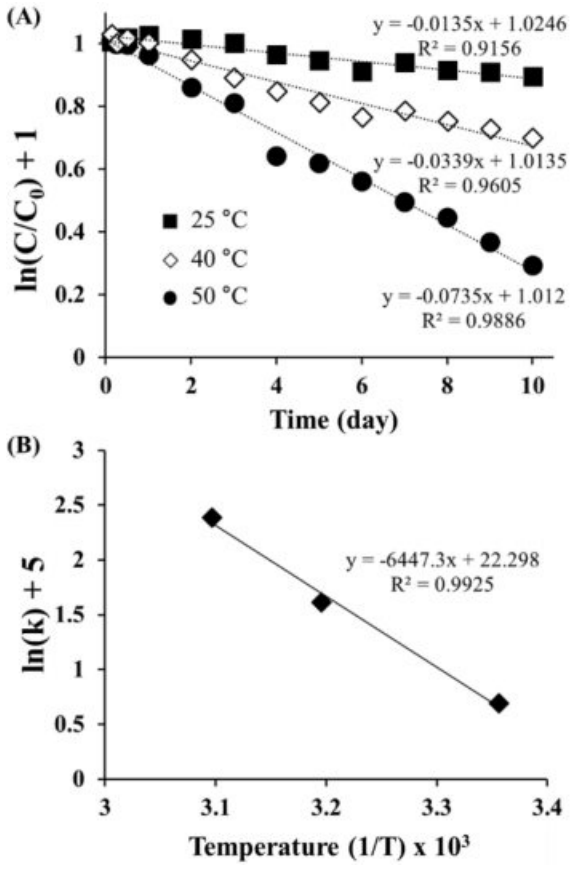

Figure 3. Kinetic analyses of ORG degradation: (A) First-order plots of remaining ORG in ASEx solution at different temperatures; (B) Arrhenius plot of the degradation rate constant *versus* the reciprocal absolute temperature.

is influenced by temperature.[35] The difference in the E_a values suggests that ORG is more stable than HST, possibly due to its glycosylated structure.

The instability of these polyphenolic compounds can be further explained by structural comparisons as depicted in Figure 4. As marked by arrows, the 3,4-dihydroxyphenyl groups are commonly prone to oxidation. Moreover, the existence of any double bonds in an aliphatic hydrocarbon chain could accelerate oxidative degradation. As a representative, HST is extremely unstable due to both the 3,4-dihydroxyphenyl group and double bonds within its structure.[36] Various antioxidants including ascorbic acid, sodium edetate, and ascorbyl palmitate have been screened to overcome the instability of HST, but no stability enhancement has been practically attained.[34] Numerous studies have shown that, due to its structural similarity to HST, curcumin is degraded by double-bond oxidation.[37] However, in the case of ORG, which possesses a glucopyranoside, aliphatic chains are saturated and subsequently stabilized. There is a general consensus that glycosylation increases the stability of polyphenols during ambient storage, even against exposure to oxygen, pH imbalances, temperature and/or light, while also enhancing gastrointestinal absorption.[38-40] Nevertheless, ORG is still unstable in aqueous solution, and its degradation is accelerated with increasing temperature. ORG degradation might be attributed to oxidation and/or enzymatic hydrolysis. As hydrolytic degradation requires presence of glycosidase, ORG degradation in enzyme-free ASEx solutions should instead occur via oxidative pathways. In comparison, quercetin is a labile compound that belongs to the polyphenol class but does not include sugar moieties or aliphatic chains. Quercetin has been reported to undergo thermally accelerated oxidative degradation in aqueous solutions with a half-life of 0.08 days at 37 °C.[41] Therefore, we suggest that ORG degradation is primarily mediated by oxidation of the dihydroxyphenyl groups.

Accelerated Stability Assessment of Ointments. The stability of ASEx-containing ointments was evaluated under an accelerated condition of 40 °C for 120 days. As shown in Figure 5, linearity was observed in the semi-log plot of the remaining level of ASEx as a function of time, indicating that ASEx degradation in the ointments followed first-order kinetics. In comparison to the PEG-based ointments, white petrolatum-based ointments showed a rapid decrease in ASEx content. The calculated rate constants and half-lives are listed in Table 6. The half-lives of PEG-based ointments were in the range of 400 to 3460 days, much longer than those of white

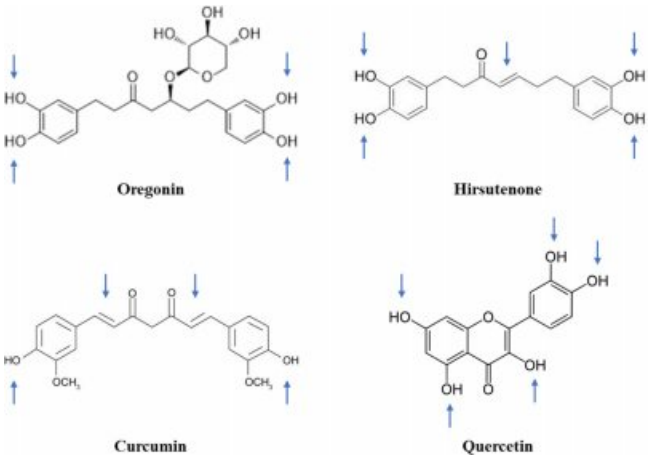

Figure 4. Comparison of the structural similarity of polyphenolic compounds. Blue-colored arrows indicate the possible points of degradation in the molecules.

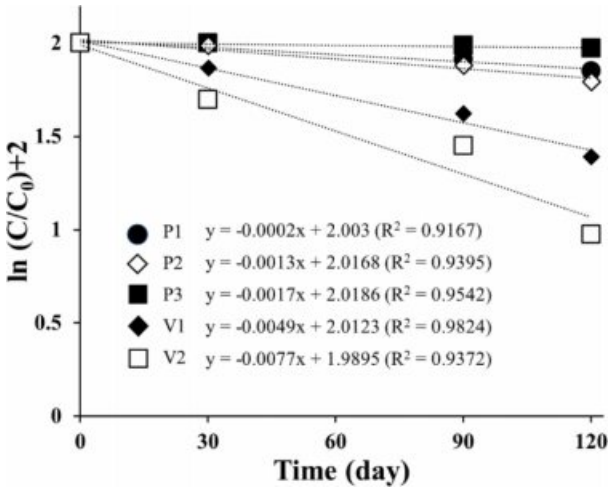

Figure 5. Degradation of ORG in ointment formulations during storage under accelerated conditions at 40 °C.

petrolatum-based ointments ranging from 90 to 140 days. In the literature, the PEG molecule can act as both a hydrogen donor and acceptor.[42] Hydrogen bonds between PEG and hydroxyl group-rich molecules such as felodipine, polyvinyl alcohol, and chitosan have been widely reported.[43-45] In addition, polyphenolic compounds such as ORG have also been found to contribute to hydrogen bonding.[46] Thus, we assumed the presence of a hydrogen bond between ORG and PEG could consequently improve the stability of ORG in ASEx-containing PEG-based ointments. This stabilization effect might be related to the changes in the consistency of the ointments during the acceleration tests. As shown in Figure 6, after 120 days of storage under the accelerated condition, changes in color and apparent consistency were observed. In the beginning (day 0), all samples exhibited a semisolid property with a yellowish-brown color that reflected the inherent color of ASEx, regardless of ointment type. However, at day 120, all ointments except P3 demonstrated physical instability through liquefaction and/or melt-down in P1 and P2, phase separation in V1 and V2, and dark brown color changes in P1, P2, V1, and V2. The color changes may be an indirect indicator of ASEx degradation. The ointment matrices with low viscosity allow for greater molecular movement, consequently providing more chances for molecular aggregation and/or collisions to facilitate reactions.[47] P3 showed much greater stability compared to the other ointments and exhibited higher viscosity than either P1 or P2. This peculiar stability may be attributed to not only the hydrogen bond formation between ORG and PEG molecules, but also complex formation induced by the zinc stearate.

We assumed that PEG molecules could form a complex with ORG and zinc stearate via hydrogen bonding to increase ORG stability, along with the viscosity of PEG-based ointments. Thus, to further investigate the effects of zinc stearate, P3 compositions were formulated with different contents of zinc stearate or sodium stearate. As shown in Figure 7(A), viscosity was increased as the amount of zinc stearate or sodium stearate increased. High concentrations of solid particles provide stiffness to semi-solid formulations.[48] With additions up to 3%, no noticeable differences were observed between sodium stearate and zinc stearate. Beyond 3%, sodium stearate moderately increased viscosity, whereas steep increases in viscosity values occurred with zinc stearate to approximately 190 Pa·s (5%), 230 Pa·s (7%), and 430 Pa·s (10%). Due to its divalent cation nature, zinc stearate has a cone-shaped structure and can offer

Table 6. Degradation Kinetics of ASEx in Ointments under the Acceleration Test

Parameter	P1	P2	P3	V1	V2
Rate constant ($\times 10^{-2}$, day^{-1})	0.13	0.17	0.02	0.49	0.77
Half-life (day)	533.19	407.73	3465.74	141.46	90.02
Shelf life (day)	81.05	61.98	526.80	21.50	13.68

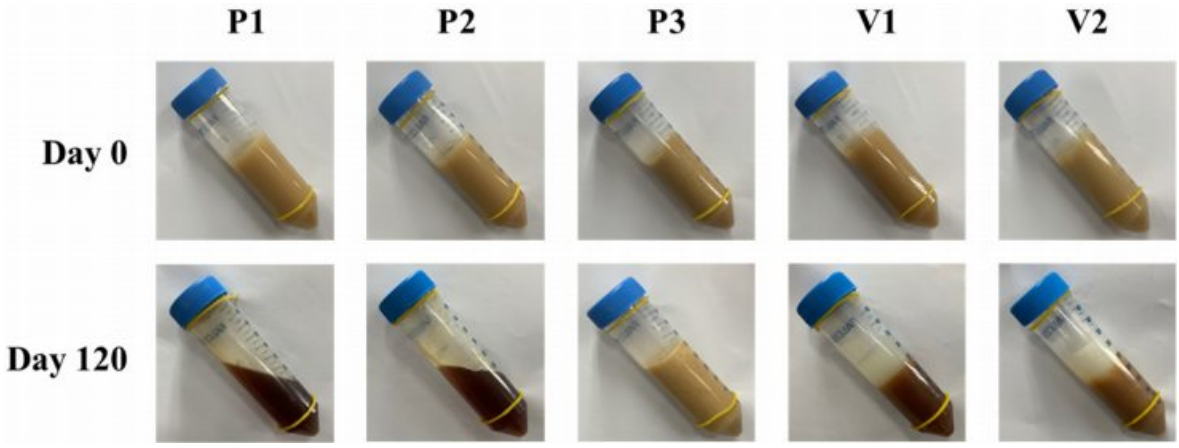

Figure 6. Changes in apparent consistency assessment of the different ointments during storage under accelerated conditions at 40 °C for 120 days.

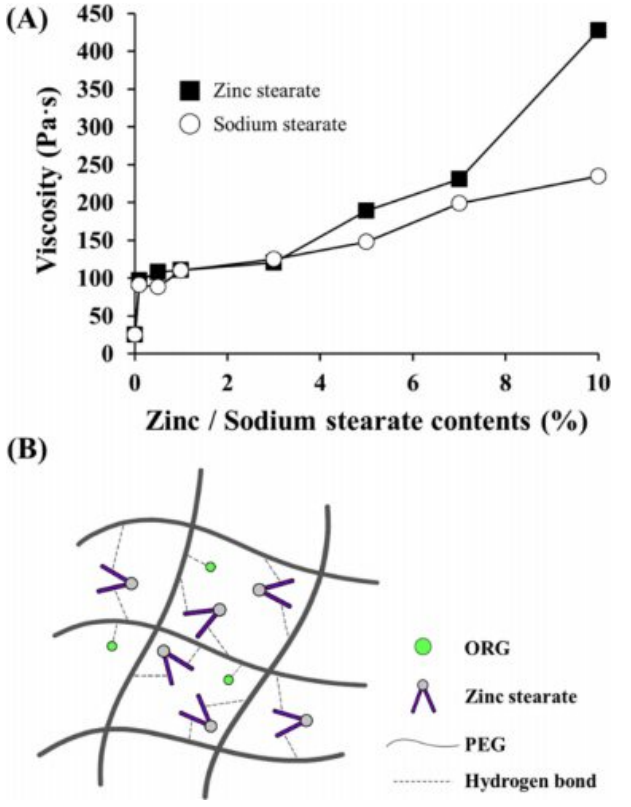

Figure 7. Effects of zinc stearate on the viscosity and network structure of the P3 ointment: (A) Viscosity changes as a function of zinc stearate and sodium stearate concentrations (0.1~10%); (B) Schematic illustration of a cross-bridge network structure via hydrogen bonds between PEG molecules and ORG or zinc stearate.

a cross-bridged network structure as depicted in Figure 7(B), subsequently increasing viscosity. In comparison, sodium stearate provides only a linear-structured linkage, which does not increase viscosity. Reversible macromolecular interactions such as hydrogen bonding can generate a networked matrix structure,[49] and hydrogen bonds between stearic acid and PEG molecules were found to increase viscosity.[50] When viscosity exceeds 200 Pa·s, a very stiff and less spreadable ointment is formed,[51] which might be disadvantageous for topical application. In this regard, P3 formulations that contain 5% zinc stearate may be a suitable for developing an ointment with improved ASEx stability.

Shelf Life Estimation Using the Q_{10} Method. Based on the accelerated stability analysis of ASEx in ointments, the expected shelf lives at room (25 °C) and refrigerated (4 °C) temperatures were calculated by applying the Q_{10} rule. The Q_{10} rule is a simple approach to predict the valid shelf life at different temperatures by using eq. (4):

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{\left(\frac{\Delta T}{10}\right)}} \quad (4)$$

where $t_{90}(T_2)$ is the predicted shelf life at temperature T_2, $t_{90}(T_1)$ is the observed shelf life at temperature T_1, and Q_{10} is a rate constant quotient related to activation energy.[52] For pharmaceutical predictions, the value of Q_{10} is commonly set at 2, 3, or 4 because these correspond to a reasonable activation energy. Because the reaction rate increases as the temperature is raised by ten degrees, the Q_{10} value is calculated by eq. (5):

$$Q_{10} = k_{T+10}/k_T \quad (5)$$

where k is a rate constant at a specified temperature. In this experiment, by introducing the rate constants of ORG deg-

Table 7. Estimated Shelf Lives of ASEx-containing Ointments at Different Temperatures

Temperature	P1	P2	P3	V1	V2
25 °C	259.07	198.12	1683.98	68.73	43.74
4 °C	1318.26	1008.05	8568.46	349.73	222.56

Values represent the shelf life (days) calculated by employing the Q_{10} value of 2.17.

radation in ASEx solutions at 40 °C and 50 °C, the Q_{10} value was calculated as 2.17. With this value, the shelf lives of the different ointment formulations were calculated (Table 7). The results suggested that the shelf lives of PEG-based ointments were longer than those of white petrolatum-based ointments. Specifically, P3 showed the longest shelf life of more than four years at 25 °C while other ointments resulted in a shelf life of less than a year. However, under a refrigerated storage condition, P1 and P2 demonstrated shelf lives of more than two years, indicating the potential for development of a commodity. In contrast, V1 and V2 revealed shelf lives of less than one year even when stored at 4 °C, indicating inadequate storage stability. Thus, P3 would be the preferable option for the development of a commercial product with sufficient long-term stability.

The Q_{10} rule is commonly used to estimate the shelf life of drugs in solutions or drug powders for which the degradation process is temperature-dependent.[53] Although accelerated stability assessments can be applied to ointment formulations, there is no assurance that the obtained results can be extrapolated to different storage conditions or quantitative expiry dating. Changes in temperature may alter other factors that affect drug stability. For example, the crystallization and viscosity characteristics of emulsions change as temperature increases.[54] Thus, further studies are necessary not only to observe any potential changes in physicochemical stability but also to determine *in vivo* efficacy for atopic dermatitis treatment.

Conclusions

ASEx-containing ointments were successfully formulated in the presence or absence of zinc stearate as a stabilizer. The rheological behavior of both the PEG-based and white petrolatum-based ointments displayed a shear-thinning system, indicating good flow properties for topical application. PEG molecules are able to form complexes with ORG and zinc stearate via hydrogen bonding, improving ASEx stability and providing high viscosity to the ointment matrix. Based on the Q_{10} method, the shelf life of P3 was estimated to be more than four years at 25 °C, suggesting that the P3 formulation containing 5% zinc stearate may be a suitable candidate for topical formulations with improved stability of ASEx. However, further studies are needed to evaluate the formulation's *in vivo* efficacy for the treatment of atopic dermatitis.

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