

Formulation of a topical cream from *Heliotropium foertherianum* extract for potential application in the supportive treatment of jellyfish sting-induced skin injuries

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ABSTRACT

Heliotropium foertherianum leaves are a rich source of rosmarinic acid, a phenolic compound with promising biological activities. This study aimed to develop a topical cream containing a rosmarinic acid-rich extract from *H. foertherianum* leaves and establish its quality specifications. Cream formulations were prepared by direct emulsification using different concentrations of oil phase and emulsifiers. The physicochemical properties of the formulations, including appearance, homogeneity, physical stability, washability, spreadability, and pH, were evaluated to identify the optimal composition. The selected formulation consisted of 75% aqueous phase, 20% oil phase, and 5% Aquagel 35S. The cream showed good physical characteristics, satisfactory stability, a spreadability of 39,85 cm², and a pH value of 6.47. Quality specifications were established and successfully verified in three independent batches. Rosmarinic acid was quantified by HPLC and used as a marker compound for quality control. The developed formulation provides a suitable basis for further studies on the biological activity.

Keywords: *Heliotropium foertherianum*; Rosmarinic acid; Topical cream.

1. INTRODUCTION

Jellyfish sting-induced skin injuries are among the most common marine-related accidents, particularly affecting fishermen, naval personnel, and individuals who frequently work or engage in activities at sea. Toxins released from jellyfish tentacles can cause pain, erythema, edema, and dermatitis, and in some cases may lead to secondary infections or delayed wound healing. Current management strategies primarily focus on pain relief, anti-inflammatory therapy, and the control of allergic reactions [1]. However, the availability of specialised topical formulations for treating jellyfish sting-induced skin injuries remains limited, particularly in Vietnam. *Heliotropium foertherianum* Diane & Hilger is a coastal plant species widely distributed in tropical shorelines and island ecosystems. In traditional medicine, the leaves have been used to treat jellyfish stings, snakebites, and ciguatoxin-related fish poisoning [2]. Phytochemical investigations have revealed that *H. foertherianum* is rich in biologically active phenolic compounds, among which rosmarinic acid is the major bioactive constituent and a characteristic marker compound of the species. In addition, the leaves contain flavonoids and other natural antioxidants [3]. Pharmacological studies have demonstrated that leaf extracts of *H. foertherianum* and rosmarinic acid possess a wide range of biological activities, including antioxidant, anti-inflammatory, neuroprotective, and detoxifying effects [4]. Notably, rosmarinic acid has been reported to inhibit hemorrhagic activity induced by various snake venoms by suppressing proteolytic enzymes, preventing vascular basement membrane degradation, and reducing tissue damage [5]. Furthermore, it has been shown to protect endothelial cells, reduce edema formation, and attenuate inflammatory cell infiltration in

experimental models. Leaf extracts and rosmarinic acid have also demonstrated the ability to counteract the toxic effects of ciguatoxin, a common marine toxin associated with fish poisoning [6]. Owing to its anti-inflammatory, antioxidant, detoxifying, and tissue-protective properties, *H. foertherianum* represents a promising natural resource for the development of topical formulations intended to support the treatment of skin injuries caused by marine toxins. In addition, Marafon et al. (2019) reported that a nanoemulsion-based hydrogel containing rosmarinic acid exhibited suitable physicochemical properties for topical application, provided sustained drug release, enhanced skin retention of rosmarinic acid, and demonstrated good biocompatibility in HaCaT keratinocytes, highlighting its potential use in skin care and tissue repair formulations [7].

Despite the recognized pharmacological potential of *H. foertherianum*, studies focusing on the development of topical formulations containing its leaf extract for the management of jellyfish sting-induced skin injuries remain scarce. Therefore, the present study aimed to formulate a topical cream containing a rosmarinic acid-rich extract from *H. foertherianum* leaves and to evaluate its physicochemical characteristics. The findings are expected to provide a scientific basis for the development of herbal-based topical products for the supportive treatment of jellyfish sting-induced skin injuries

2. MATERIALS AND METHODS

2.1. Materials

A bioactive-rich extract obtained from *H. foertherianum* leaves containing more than 80% rosmarinic acid. Leaves of *Heliotropium foertherianum* were collected from the Truong Sa Archipelago, Vietnam. The plant material was taxonomically identified and authenticated by the Institute of Biology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Excipients used for cream formulation included Tween 80, cetyl alcohol, stearic acid, vaseline, liquid paraffin, propylene glycol, glycerin, methylparaben (Nipagin), and propylparaben (Nipasol), all of pharmaceutical grade and purchased from Sigma-Aldrich. Aquagel 35S (China) was used as the emulsifying and thickening agent. Distilled water meeting pharmacopeial specifications was used throughout the study. HPLC-grade acetonitrile, formic acid, rosmarinic acid and water were purchased from Sigma-Aldrich.

2.2. Methods

2.2.1. Preparation of cream containing the bioactive-rich extract

Cream formulations were prepared using the direct emulsification method. Briefly, the emulsifier (Tween 80 or Aquagel 35S) was dispersed in the aqueous phase containing the extract, propylene glycol, glycerin, nipagin, and distilled water, then heated to 75–80 °C with continuous stirring. The oil phase, consisting of cetyl alcohol, vaseline and liquid paraffin, was heated separately to 70–75 °C. The aqueous phase was then slowly added to the oil phase under continuous stirring. The resulting emulsion was cooled to approximately 25 °C, and the pH was adjusted to 4.5–7.0 when necessary [8]. Formulations were prepared with varying proportions of oil phase and emulsifiers, while maintaining constant concentrations of the extract (12%), nipagin (0.10%), nipasol (0.12%), propylene glycol (5%), glycerin (5%), and distilled water (100 mL).

The oil phase was investigated at concentrations of 10%, 15%, 20%, 25%, and 30%, consisting of vaseline (50%), liquid paraffin (35%), cetyl alcohol (10%), and stearic acid (5%). Emulsifiers including Tween 80 and Aquagel 35S were evaluated at concentrations of 1%, 3%, 5%, and 7%.

2.2.2. Evaluation of cream formulations [9]

Appearance: The formulations were evaluated for softness, smoothness, pleasant odour, pale yellow colour, and absence of air bubbles.

Physical stability: Approximately 10 g of cream was placed in a sealed test tube and subjected

to accelerated stability testing at 10 ± 1 °C and 45 ± 2 °C for 24 h. The formulations were considered stable if no visible changes or phase separation occurred.

Homogeneity: Approximately 0.02–0.03 g of cream was spread evenly between two glass slides and compressed until a circular film with a diameter of approximately 2 cm was formed. The preparation was visually examined from a distance of approximately 30 cm. The formulation was considered homogeneous when no visible particles were observed in at least three of four replicates.

Washability: Approximately 1 mL of cream was applied to the skin and gently rubbed for 10 s. The skin was subsequently rinsed under running water. Formulations were evaluated based on ease of removal and absence of residual greasiness.

Spreadability: One gram of cream was placed between two glass plates and compressed with a 1000 g weight. The diameter of the spread cream was measured in two perpendicular directions and averaged.

2.2.3. Establishment of quality specifications for the cream formulation [9]

The optimised formulation was prepared in three independent batches. Quality specifications were established based on the mean values obtained from the three batches: Appearance: soft, smooth consistency with a pleasant odor and pale yellow color (Vietnamese Pharmacopoeia V, Appendix 1.12); Weight uniformity: determined according to Vietnamese Pharmacopoeia V, Appendix 11.3; pH: 4.5–7.0; Physical stability: evaluated as described above; Homogeneity: determined according to Vietnamese Pharmacopoeia V, Appendix 1.12; Microbial limits: determined according to Vietnamese Pharmacopoeia V, Appendix 16.3; Heavy Metal Limit: determined according to AOAC 2015.1 (ICP-MS).

2.2.4. Quantification of rosmarinic acid in the cream formulation

Rosmarinic acid content was determined using an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) in accordance with the Vietnamese Pharmacopoeia V (Appendix 5.3) [9]. The analysis was performed at the Institute of Materials, Biotechnology and Environment, Hanoi, Vietnam

Chromatographic conditions: Analysis was performed on a reversed-phase C18 column (250 mm $\times$ 4.6 mm, 5 μ m). The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile). A gradient elution program from 20% to 60% solvent B over 20 min was applied. The flow rate was maintained at 1.0 mL/min, the column temperature was set at 25–30 °C, and the injection volume was 10 μ L, detection was performed at 330 nm [10].

Preparation of standard and sample solutions: The standard stock solution was prepared by accurately weighing approximately 10 mg of rosmarinic acid reference standard (purity $\geq$ 98%), dissolving it in methanol, and diluting to 10 mL to obtain a concentration of 1000 μ g/mL. Working standard solutions of 5, 10, 20, 50, and 100 μ g/mL were subsequently prepared to construct the calibration curve.

Sample preparation: approximately 50 mg of cream sample was accurately weighed into a 100 mL volumetric flask. Fifty millilitres of methanol was added, and the mixture was sonicated for 15–30 min to ensure complete extraction of rosmarinic acid. The volume was then adjusted to the mark with methanol. The resulting solution was filtered through a 0.45 μ m membrane filter prior to HPLC analysis.

3. RESULTS AND DISCUSSION

3.1. Formulation development

The results of the evaluation of formulation components and their proportions are presented in Table 1.

Table 1. Evaluation results of cream formulations.

Water phase %	Oil phase %	Emulsifier concentration (%)			
		Tween %	Results	aquagel 35S %	Results
89	10	1	Phase separation	1	Semi-solid
87		3	Phase separation	3	Semi-solid
85		5	Phase separation	5	Semi-solid
83		7	Phase separation	7	Semi-solid
84	15	1	Phase separation	1	Semi-solid
82		3	Phase separation	3	Semi-solid
80		5	Phase separation	5	Semi-solid
78		7	Phase separation	7	Semi-solid
79	20	1	Semi-solid	1	Semi-solid
77		3	Semi-solid	3	Semi-solid
75		5	Semi-solid	5	Smooth cream
73		7	Semi-solid	7	Smooth cream
74	25	1	Phase separation	1	Semi-solid
72		3	Homogeneous	3	Semi-solid
70		5	Phase separation	5	Smooth cream
68		7	Phase separation	7	Smooth cream
69	30	1	Homogeneous	1	Semi-solid
67		3	Phase separation	3	Semi-solid
65		5	Phase separation	5	Smooth cream
63		7	Phase separation	7	Smooth cream

Preliminary screening of emulsion formulations was conducted by varying the oil phase content (10–30%) and emulsifier concentration (1–7%). Two emulsifying systems, Tween and Aquagel 35S, were evaluated for their ability to produce stable cream formulations.

Formulations prepared with Tween exhibited poor emulsion stability. Most formulations showed phase separation during preparation or shortly thereafter, regardless of the oil phase concentration. Although phase separation was not observed in formulations containing 25% oil phase with 3% Tween and 30% oil phase with 1% Tween, these systems did not exhibit desirable cream characteristics and remained semi-liquid. These findings indicate that Tween alone was insufficient to stabilise the emulsion system under the investigated conditions.

In contrast, formulations containing Aquagel 35S demonstrated improved rheological properties and physical stability. At concentrations of 1–3%, the resulting products remained semi-liquid and lacked the consistency required for topical cream applications. Increasing the Aquagel 35S concentration to 5–7% markedly improved the texture of the formulations, producing homogeneous, smooth creams when combined with oil-phase concentrations ranging from 20% to 30%. No visible phase separation was observed in these formulations during the initial evaluation.

The results suggest that both the oil phase content and emulsifier concentration significantly influenced cream formation. An oil phase concentration below 20% was insufficient to develop a stable cream structure, whereas Aquagel 35S concentrations of at least 5% were required to achieve appropriate viscosity and emulsion stability. Increasing the Aquagel 35S concentration from 5% to 7% did not provide a noticeable improvement in the macroscopic appearance of the formulations.

The preliminary screening results indicated that formulations containing 20–30% oil phase and 5% Aquagel 35S exhibited favourable physicochemical properties and were therefore selected for further optimisation. According to the manufacturer's technical documentation, Aquagel 35S is typically recommended at concentrations of 2–5% for preparing oil-in-water emulsions and topical cream systems.

Subsequently, three formulations were developed with aqueous phase contents ranging from 65% to 75% and corresponding oil phase contents of 20–30%, while maintaining Aquagel 35S at a fixed concentration of 5%. The formulations were further evaluated for properties, as shown in Table 2.

Table 2. Physicochemical evaluation of cream formulations with varying oil phase content.

Parameters	CT1	CT2	CT3	Parameters	CT1	CT2	CT3
Aqueous phase (%)	75	70	65	Homogeneity	+	+	+
Oil phase (%)	20	25	30	Washability	+	+	+
Aquagel 35S (%)	5	5	5	Spreadability (cm ²)	39.85	35.84	34.93
Appearance	+	+	+	pH	6.47	6.51	6.59
Physical stability	+	+	+				

All investigated formulations (CT1–CT3) exhibited acceptable physicochemical characteristics, including a smooth appearance, homogeneous texture, and satisfactory physical stability without observable phase separation during the preliminary evaluation. These findings indicate that Aquagel 35S at a concentration of 5% provided sufficient emulsifying capacity to establish and maintain a stable emulsion system over a broad range of oil-phase contents (20–30%).



Figure 1. Macroscopic appearance of the cream formulation (CT1, CT2 and CT3).

Despite the comparable stability profiles, variations in the oil-to-water ratio significantly influenced the spreading behaviour of the formulations. A gradual decrease in spreadability was observed as the oil-phase content increased, with CT1, CT2, and CT3 exhibiting spreadability values of 39.85, 35.84, and 34.93 cm², respectively. This trend is likely associated with the increasing proportion of lipophilic excipients, including petrolatum, liquid paraffin, cetyl alcohol, and stearic acid, which contribute to higher viscosity and stronger structural interactions within the emulsion network. Consequently, the cream's resistance to flow under an applied force increased, resulting in reduced spreadability.

The higher aqueous-phase content of CT1 contributed to a less viscous system, facilitating easier spreading on the skin surface. Enhanced spreadability is generally considered desirable for topical formulations because it promotes uniform distribution of active ingredients, improves ease of application, and may increase patient acceptability. Although formulations containing higher oil-phase levels may provide improved emollient and occlusive effects, excessive oil content can lead to a greasier skin feel and reduced spreading performance.

Considering that all formulations met the predefined criteria for appearance, homogeneity, washability, pH, and physical stability, spreadability emerged as the primary factor differentiating formulation performance. Therefore, CT1 was selected as the optimal base formulation for subsequent studies. In addition to exhibiting the highest spreadability, CT1 contained the lowest oil-phase concentration (20%), which may improve sensory characteristics while maintaining adequate formulation stability. These results further demonstrate the effectiveness of Aquagel 35S as an emulsifying system, capable of producing a stable cream even at relatively low oil-phase levels.

3.2. Establishment of quality specifications for the topical cream

The average quality control results obtained from three batches of the cream formulation are shown in Table 3.

Table 3. Mean quality control results of three batches of cream.

No.	Quality attribute	Specification	Result
1	Appearance	Homogeneous, smooth cream with a light yellow to light brownish-yellow color, characteristic odor, and no phase separation	Pass
2	Weight uniformity	All units must have a weight within $\pm 10\%$ of the labeled weight	Pass
3	pH	pH range of 4.5 - 7.0	6.47
4	Physical stability	No evidence of phase separation, aggregation, or changes in appearance after accelerated stability testing	Pass
5	Homogeneity	Uniform preparation with no visible coarse particles or lumps	Pass
6	Spreadability (cm ²)	Spreadability area ≥ 30 cm ²	39.85
7	Washability	Easily washable with water and leaves no significant oily residue on the skin surface	Pass
8	Rosmarinic acid (%)	$\geq 8\%$	9,01
9	Heavy metal limit (ppm)	< 10 ppm	Pass
10	Microbial limit	Absence of total aerobic bacteria, total fungi, <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , and <i>Candida albicans</i> in 1 g of sample	Pass

The theoretical rosmarinic acid content in the cream was estimated at approximately 9.6% (w/w), based on incorporating 12% extract containing more than 80% rosmarinic acid. The experimentally determined content was 9.01%, corresponding to approximately 93.9% of the theoretical value. This slight reduction may be attributed to several factors, including incomplete extraction of rosmarinic acid from the cream matrix during HPLC sample preparation, minor losses during manufacturing, and analytical variability. Although the cream was prepared at approximately 80 °C, rosmarinic acid has been reported to exhibit relatively good thermal stability under short-term heating conditions; therefore, thermal degradation is unlikely to be the primary cause of the observed difference. Overall, the measured rosmarinic acid content remained close to the theoretical value, indicating that the manufacturing process effectively preserved the marker compound. The consistency of the quality control results across three independent batches demonstrated good reproducibility of the manufacturing process, indicating that the developed cream formulation is suitable for further biological evaluation

4. CONCLUSIONS

A topical cream containing a rosmarinic acid-rich *Heliotropium foertherianum* extract was successfully developed using 75% aqueous phase, 20% oil phase, and 5% Aquagel 35S. The optimized formulation exhibited desirable physicochemical properties, including a homogeneous and smooth appearance, satisfactory washability, good spreadability, and physical stability without phase separation. The cream also showed a skin-compatible pH value 6.47, suggesting suitability for topical application. Quality evaluation of three independent batches confirmed good manufacturing reproducibility and compliance with all established specifications, including weight uniformity, rosmarinic acid content, heavy metal limits, and microbiological requirements. Overall, the developed cream formulation exhibited suitable physicochemical properties and met

the predefined quality attributes, demonstrating its feasibility as a topical formulation containing *Heliotropium foertherianum* extract.

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TÓM TẮT

Nghiên cứu bào chế kem bôi da từ cao chiết lá cây phong ba (*Heliotropium foertherianum*) định hướng hỗ trợ điều trị vết thương do sứa

Nghiên cứu này, nghiên cứu bào chế kem bôi da chứa cao chiết lá cây phong ba giàu acid rosmarinic và xây dựng tiêu chuẩn chất lượng cho chế phẩm. Các công thức kem được bào chế bằng phương pháp nhũ hóa trực tiếp với các nồng độ pha dầu, pha nước và chất nhũ hóa khác nhau. Đặc tính lý hóa của kem, cảm quan, độ đồng nhất, độ ổn định vật lý, khả năng rửa trôi, độ đàn mỏng và pH, được đánh giá nhằm lựa chọn công thức tối ưu. Công thức được lựa chọn gồm 75% pha nước, 20% pha dầu và 5% Aquagel 35S. Kem thu được có đặc tính cảm quan tốt, độ ổn định đạt yêu cầu, độ đàn mỏng 39,85 cm² và pH 6,47. Tiêu chuẩn chất lượng của chế phẩm kem bôi đã được xây dựng và kiểm định thành công trên ba lô sản xuất độc lập. Công thức kem bào chế có các đặc tính lý hóa và chỉ tiêu chất lượng phù hợp, tạo cơ sở cho các nghiên cứu tiếp theo nhằm đánh giá hoạt tính sinh học.

Từ khoá: *Heliotropium foertherianum*; Acid rosmarinic; Kem bôi da.