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FORMULATION AND STABILITY EVALUATION OF CREAM CONTAINING *CHROMOLAENA ODORATA* AND *CENTELLA ASIATICA* LEAF EXTRACTS

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ABSTRACT: *Chromolaena odorata* was extracted by ethanol/water. *Centella asiatica* was extracted by methanol. A cream containing *C. odorata* and *C. asiatica* extracts was formulated and evaluated for its physicochemical properties and stability. *C. odorata* and *C. asiatica* were extracted and their respective active compounds scutellarein tetramethyl ether and asiaticoside were determined as bioactive compounds using HPLC. Creams were prepared using 5% *C. odorata* and 1% *C. asiatica* extracts and tested for their appearance, pH, and viscosity. Stability was evaluated under accelerated conditions using six hot/cold cycles and after long-term storage at 30 °C/75% RH for 3 months. The content of scutellarein tetramethyl ether in *C. odorata* extract was 0.13 ± 0.02% w/w and the content of asiaticoside in the *C. asiatica* extract was 0.35 ± 0.04% w/w. Contents of scutellarein tetramethyl ether and asiaticoside in the cream formulation were 98.25 ± 0.43% and 102.16 ± 0.22%, respectively. The cream formulations containing the extracts had a good appearance and were non-greasy and easily applied, with a pH value of 5.4 ± 0.1 and viscosity of 1604 ± 257 poises determined at shear rate 0.08 s^{-1}. No changes in physical and chemical properties occurred during storage. These findings indicate that this formulation of 5% *C. odorata* and 1% *C. asiatica* extract is stable and suitable for further testing as a topically applied product.

INTRODUCTION: Siam weed (*Chromolaena odorata*) is a perennial scandent or semi woody shrub in the Asteraceae family which has been used historically for a variety of ailments in tropical countries, especially to stop bleeding.

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Studies have demonstrated that Siam weed extract accelerates hemostasis and wound healing [1-4], and contains various compounds with pharmaceutical potential [4, 5] including beta-caryophyllene, humulene, stigmasterol, and scutellarein tetramethyl ether [2, 5, 6]. Several literatures provide information on preliminary phytochemical screening, antibacterial activity, and antioxidant activity of *C. odorata* leaf extracts [7-11]. Scutellarein tetramethyl ether, in particular, is useful for standardization of this plant extract [6] and has been reported as a bioactive component for blood coagulation.

We chose this compound as an active ingredient for cream formulation quality control. Meanwhile, *Centella asiatica* belongs to family Apiaceae (Umbeliferae) with distribution in India, Southeast Asia, and the USA and is a popular traditional medicinal plant used in wound healing [12, 13]. It contains biologically active compounds such as asiatic acid, madecassic acid, asiaticoside, and madecassoside [12, 14]. Due to its ability to stimulate collagen synthesis, *C. asiatica* has been used in cream, gel, or topical spray formulations for restoring skin firmness and elasticity and improving its appearance [12, 15]. In addition, there are reports that both *C. asiatica* and *C. odorata* have antibacterial and antioxidant activities [3, 13]. We chose asiaticoside as an assay target for evaluating *C. asiatica* extract because of its detectable content and strong effect on wound healing.

Both plants are commonly found in Thailand but do not presently have agricultural worth. No reports have been published on a combination of these two plants in a topical cream formulation. Creams are semisolid dosing formats intended mainly for external use, which generally consists of an oily internal phase and an aqueous external phase. Due to the emulsified nature of skin surface, drugs formulated as cream more effectively interact with skin and more readily penetrate through biological membranes [16]. As such, we aimed to develop a skin cream containing both *C. odorata* and *C. asiatica* leaf extracts in order to treat wounds via topical application to the locally damaged tissue. Our study aimed to determine the content of active compounds in *C. odorata* and *C. asiatica* leaf extracts and to prepare a cream containing both. We evaluated our formulations based on their physicochemical properties and physical and chemical stabilities.

MATERIALS AND METHODS:

Materials: Scutellarein tetramethyl ether (95.20%) was obtained from Chengdu GLP Biotech Co., Ltd. (Sichuan, China). Asiaticoside (≥98%) were obtained Fluka Chemie GmbH (Switzerland). Nicotinamide was procured from Hangzhou Toyond Biotech Co. Ltd (China). Panthenol, mineral oil and castor oil were purchased from Srichand United Dispensary Co. Ltd (Thailand). Sorbitan stearate 60, polysorbate 60, glycerine, isopropyl myristate, glyceryl monostearate, cetostearyl alcohol, and tocopherol acetate were supplied from P.C. Drug (Thailand). And all other ingredients are of pharmaceutical grade. Monobasic potassium phosphate was obtained from Merck, Darmstadt, Germany. Petroleum ether, methanol, ethanol, and acetonitrile was purchased from RCI Labscan (Thailand). All other chemicals were of pharmaceutical grade and were used as received.

Extraction and Analysis of *C. odorata*: Leaves of *C. odorata* were collected from Walailak University campus in Nakhon Si Thammarat Province in the south of Thailand. *C. odorata* extract was prepared according to a previously published method [9]. Briefly, leaves were washed and air-dried in shade, cut into small pieces, powdered using an automatic grinder, and passed through a #40 mesh sieve, then stored in a closed vessel. To prepare the extract, 500 g of powder was extracted in 70% v/v ethanol (1:10 w/v) at 25 °C and shaken at 120 rpm for 12 h. The mixture was filtered through Whatman filter paper no.1, and the filtrate was concentrated using a rotary evaporator at 60 °C. The dark green viscous extract was separately kept in tightly closed brown vials at 4 °C until used. The extract yield was 10.22% w/w with respect to dry powdered plant material.

Quantitative analysis of scutellarein tetramethyl ether in this extract was carried out using high-performance liquid chromatography (HPLC) (Ultimate 3000, Dionex Corporation, USA), using 95.2% scutellarein tetramethyl ether (Chengdu GLP Biotech Co., Ltd., Sichuan, China) as a standard for comparison. The HPLC system consisted of a solvent delivery pump equipped with an in-line degasser plus a sample loop with an injection volume of 20 µL with three injections. The separation was carried out using a C18-column stainless steel (Inertsil® ODS-3, GL Sciences) eluted with methanol and 0.5 % acetic acid (60:40) with a flow rate of 1 mL/min and detection at UV 268 nm.

Extraction and Analysis of *C. asiatica*: Whole *C. asiatica* plants were collected from Songkhla Province, Thailand. The extract was prepared according to a previously reported method [12]. In brief, the aboveground plant tissues were cleaned with deionized water and air-dried, then cut into

pieces, powdered with an automatic grinder, and passed through #40 mesh sieves, then stored in a closed vessel. The powder was defatted with petroleum ether and extracted with methanol in a Soxhlet apparatus for 12 h. Methanol extraction was continued until the solvent appeared colorless. Following this, the extract was concentrated over a water bath at 50 °C and dried at 50 °C in a hot oven at atmospheric pressure. Traces of methanol were removed under reduced pressure (~8 mbar) at 60 °C using a rotary evaporator. The extract yield was 6.98% w/w with respect to dry powdered plant material.

Triterpenes in the extract were analyzed using HPLC (Ultimate 3000, Dionex Corporation, USA) [17]. An asiaticoside reference standard (≥ 98%; Fluka Chemie GmbH, Switzerland) was prepared as a stock solution in methanol and diluted further to obtain the concentration of 50-150 µg/mL. The sample extract was dissolved in 1 mg/mL methanol and filtered through a 0.45 µm nylon membrane syringe filter. The HPLC system consisted of a solvent delivery pump equipped with an in-line degasser and a sample loop with an injection volume of 20 µL with three injections. Separations were performed on a reversed-phase C-18 stainless steel column (Inertsil® ODS-3, GL Sciences) and maintained at 25 °C. The mobile phase used for the separation was 0.01 M monobasic potassium phosphate (KH_2PO_4) adjusted to pH 3 with orthophosphoric acid and acetonitrile in a ratio of 1:1 v/v. The flow rate was maintained at 1.0 mL/min, and the separation was monitored using UV at a wavelength of 205 nm.

Formulation Development and Optimization: Liquids extracts of *C. odorata* and *C. asiatica* were used for the preparation of creams for the treatment of excision wounds. *C. odorata* extract was used at 5% in this formulation, as per Yahya and Ramlee [2, 18]. *C. asiatica* extract was used at 1% according to the study of topical spray that confirms the efficacy of wound healing in animal model [12]. The cream base ingredients, including emulsifying agents, thickening agents, and oil phase ingredients were selected and adjusted in order to produce an optimized cream formulation **Table 1**. The optimization of the cream base was performed by trial and error to obtain a suitable cream. The cream was manufactured by the beaker method. Briefly, oil phase ingredients were combined in jojoba oil and heated to 70-80 °C. The other ingredients were dissolved in purified water and heated to 70-80 °C. The oil phase was transferred to the water phase and stirred continuously until a smooth uniform cream was obtained. Heat labile ingredients, as well as the two plant extracts, were added when the temperature decreased to around 40 °C. Finally, the cream was homogenized using a high-pressure homogenizer.

TABLE 1: FORMULA OF CREAM CONTAINING *C. ODORATA* AND *C. ASIATICA* EXTRACT

Ingredient	Function	Amount (%)
Chromolaena odorata extract	Active ingredient	5.0
Centella asiatica extract	Active ingredient	1.0
Panthenol (Pro-Vitamin B5)	Anti-inflammatory role, helping to soothe a dry, irritated skin	0.5
Nicotinamide (Vitamin B3)	Encouraging the production of natural emollients	0.5
Mineral oil	Emollient, skin conditioning agent	3.0
Sorbitan stearate 60	Emulsifier	6.0
Polysorbate 60	Emulsifier	6.0
Jojoba Oil	Moisturizing	3.0
Dimethylpolysiloxane	Emollient, skin conditioning agent	3.0
Glycerine	Humectant	2.0
Isopropyl Myristate	Skin conditioning agent, emollient	3.0
Castor Oil	Moisturizer and emollient	1.0
Glyceryl Monostearate	Thickening agent, emollient	5.0
Cetostearyl Alcohol	Thickening agent, emollient	4.0
Dimethicone	Emollient, skin conditioning agent	2.0
Tocopherol Acetate (Vitamin E)	Antioxidant	1.0
Phenoxyethanol	Preservative	1.0
Purified Water	Solvent	53.0

Physical Evaluation: All formulations at different concentrations were evaluated according to the following characteristics. The appearance was evaluated according to subjective organoleptic tests of color, smell, and softness after application on the skin. pH was determined using a digital pH meter (SevenEasy™ pH, Mettler Toledo, Switzerland). One gram of cream was dissolved in 100 mL of distilled water and stored for two hours.

The measurement of the pH of each formulation was done in triplicate. pH values are expressed in mean ± standard deviation. Viscosity was measured using a Modular Advanced Rheometer System (HAAKE MARS 60, ThermoFisher Scientific, Germany) at a shear rate of 0.08 s^{-1}. Results are expressed as mean ± standard deviation. Dye solubility was used to identify emulsion type, where 5 g prepared cream was mixed with water-soluble amaranth dye and dropped on a microscope slide, covered with a cover-slip, and observed under the microscope.

Stability: An accelerated stability study was performed using hot/cold cycles, where a 30 g sample was subjected to 40 °C/75 % RH for 48 h, followed by 2-8 °C for 48 h. This was repeated six times. The long term stability condition was carried out at 30 °C/75% RH. Appearance, pH, and viscosity were evaluated at 1 and 3 months. At the end of the study, active compounds were evaluated as:

Percentage of active compound content = Content of active compound in the formulation × 100 / Content of active compound in the extract

RESULTS AND DISCUSSION: The assay content of scutellarein tetramethyl ether in water/ethanol extract of *C. odorata* was 0.13 ± 0.02% w/w. The content of asiaticoside in methanol extract of *C. asiatica* was 0.35 ± 0.04% w/w.

Our formulation produced a soft, smooth, and non-greasy cream, suitable for the treatment of excision wounds and lesions on rough or bruised skin. Dye solubility testing yielded a continuous red phase, indicating that the emulsion is oil in water type with water in the external phase. As such, the dye will dissolve in it to give color. This is phase provides a non-greasy texture which is easily removable from the skin's surface, and which can be used externally to provide a cooling effect [19].

Measurement of the physical properties and stability of the cream formulations showed good homogeneity, slightly green color, smooth cream with characteristic odor **Table 2** and **Fig. 1**. The pH of the formulation was between 5.3-5.5, which is in the normal pH range of the skin [20]. The rheological behavior indicated that as torque increases, shear stress increases and viscosity decreases, showing shear thinning or pseudoplastic viscosity. Physical properties including appearance, viscosity, and pH were not changed after storage under hot/cold cycling.

In addition, samples stored at 30 °C/75% RH showed only a slight increase in viscosity, possibly caused by thickening due to water loss. Assayed content of the active compounds showed that scutellarein tetramethyl ether and asiaticoside were within the range of 97-100% **Table 2**, indicating good product stability.

TABLE 2: STABILITY RESULTS OF CREAM CONTAINING *C. ODORATA* AND *C. ASIATICA* EXTRACT AFTER STORAGE BY ACCELERATED CONDITION (FREEZE-THAW) AND LONG-TERM STORAGE CONDITION AT 30°C/75% RH (MEAN ± SD, N = 3)

Storage conditions	Test	Test interval		
		initial	1st month or 6th cycle of freeze-thaw	3rd months
30°C/75% RH	Appearance	Slightly green color, homogeneous cream with characteristic odor	unchanged	unchanged
	Assay of scutellarein tetramethyl ether	98.25 ± 0.43	Not test	97.15 ± 0.53
	Assay of asiaticoside	102.16 ± 0.22	Not test	99.73 ± 0.49
	pH	5.4 ± 0.1	5.3 ± 0.2	5.3 ± 0.2
	Viscosity (poise)	1604 ± 257	1811 ± 343	1960 ± 462
Freeze-Thaw	Appearance	Slightly green color, homogeneous cream with characteristic odor	unchanged	Not test
	Assay of scutellarein tetramethyl ether	98.25 ± 0.43	97.12 ± 0.78	Not test
	Assay of asiaticoside	102.16 ± 0.22	101.44 ± 1.65	Not test
	pH	5.4 ± 0.1	5.4 ± 0.1	Not test
	Viscosity (cps)	1604 ± 257	1610 ± 435	Not test

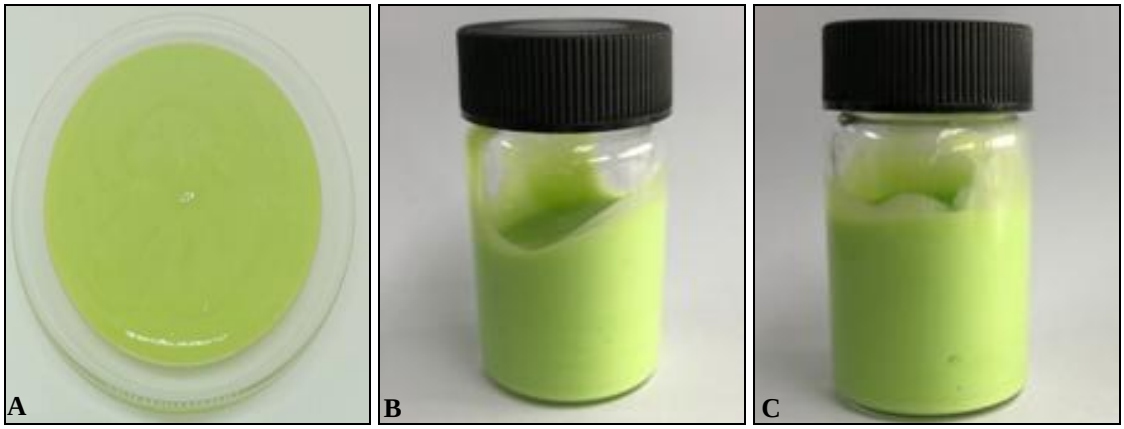

FIG. 1: EXAMPLES OF CREAM CONTAINING *C. ODORATA* AND *C. ASIATICA* EXTRACT AT INITIAL (A) AND AFTER STORAGE BY ACCELERATED CONDITION (FREEZE-THAW) (B) AND LONG-TERM STORAGE CONDITION AT 30°C/75%RH (C)

C. odorata and *C. asiatica* extracts in this formulation may be of use in treating excision wounds due to their supposed antibacterial activity and beneficial effects on wound healing and hemostasis [1, 9, 14]. Moreover, the well-known excipients such as jojoba oil, dimethylpolysiloxane, glycerine, pathenol, and isopropyl myristate (used as penetration enhancer) likely enhance its moisturizing properties and performance.

Meanwhile, the inclusion of vitamin B3 as niacinamide or nicotinamide contributes anti-inflammatory effects and improves skin appearance by reducing leucocyte peroxidase systems that may lead to localized tissue damage, as well as improving the stratum corneum barrier [21, 22]. As such, we suggest that this product is suitable for testing on excision wound models.

CONCLUSION: A topical cream formulation containing *C. odorata* and *C. asiatica* extracts was successfully developed. The physical evaluations showed acceptable of appearance, pH, and viscosity. The results indicated that this cream formulation was generally stable under stress and tropical storage conditions. To confirm the efficacy, this product will be tested on excision wound models.

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Author Contributions: Somchai Sawatdee and Apichart Atipairin have contributed to the concept, design of the study, data analysis including interpretation of data and prepare the manuscript. Somchai Sawatdee carries out of the experiment of cream preparation and stability study. Apichart Atipairin carry out of the plant extraction and analysis of active compounds.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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