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Evaluation of *In Vitro* Antioxidant Activities and Toxicity Effects of a Novel Plant-Based Body Lotion from Thai Medicinal Plants

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ABSTRACT

In addition to lessening the residue of chemicals, which are the primary ingredients in cosmetics, the hunt for herbal extracts with skin health benefits is currently highly intriguing. Local herbs are more valued as a result. This study presents the development and evaluation of a novel plant-based body lotion formulated with extracts from Thai medicinal plants, including *Stemona tuberosa*. The research aimed to assess the antioxidant activities, toxicity effects, and sensory characteristics of the lotion. Antioxidant properties were determined using 1,1-diphenyl-2-picrylhydrazyl)DPPH(, total phenolic content)TPC(, and ferric-reducing antioxidant power)FRAP(assays, revealing significant antioxidant capabilities, particularly for the *Stemona tuberosa* extract. Toxicity was evaluated using *Galleria mellonella* larvae, demonstrating minimal adverse effects in a lotion containing various concentrations of the extracts. The most promising lotion's formulation incorporated different concentrations of *Stemona tuberosa* extract (1, 3, and 5% w/w), with other ingredients including stearic acid, isopropyl myristate, and glyceryl monostearate, ensuring stability and homogeneity. Sensory evaluations were conducted employing a semi-trained panel of 40 judges using a 5-point hedonic scale, focusing on characteristics such as color, fragrance, texture, and overall acceptability. The lotion displayed favorable sensory properties, with different concentrations yielding slightly varied consumer preferences. Overall, the results indicate that a novel herbal lotion formulated with *Stemona tuberosa* is a promising candidate skincare product, offering antioxidant benefits and consumer acceptability while maintaining low toxicity.

***Keywords*:** antioxidant; toxicity; *Stemona tuberosa*; body lotion.

Introduction

The concept of using a polyherbal formulation involves combining multiple herbs in a specific ratio to create a remedy or treatment that can be employed not only in traditional Thai medicine but also in traditional Chinese,[1-4] Ayurvedic,[5] Siddha,[6] Unani,[7] and southern African traditional medicinal products.[8] The key concept is that when herbs are used together, they synergistically improve healing properties, leading to a more substantial therapeutic effect than when used individually. The purpose of using multiple herbs in a single formulation is that each herb has a unique function. Certain herbs are chosen to address the main health concern, while others are included to alleviate potential side effects or to contribute to general health, thereby leading to a more balanced and holistic treatment.[9] Additionally, the potential for adverse effects may be reduced by combining herbs. Certain herbs are explicitly included for their capacity to neutralize potential side effects from others, thus enhancing overall treatment safety.[1,9] Polyherbal formulations represent a complex, synergistic, and holistic approach to herbal medicine, deeply rooted in traditional knowledge and increasingly supported by modern scientific research.

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In Thailand, several polyherbal formulations have been used for treating various diseases, including skin infections.[10] In the present work, a polyherbal formulation for treating skin diseases, known as THR-01, consisting of *Rhinacanthus nasutus* (Acanthaceae), *Quisqualis indica* (Combretaceae), *Ocimum sanctum* (Lamiaceae), *Vitex glabrata* (Verbenaceae), and *Stemona tuberosa* (Stemonaceae) was chosen for testing its antioxidant activities and safety. Interestingly, *Stemona tuberosa*, one of the primary components of this formulation was found to exhibit significant antioxidant properties with low toxicity. Plant roots of the *Stemona* genus, part of the Stemonaceae family, have historically been a part of traditional Chinese medicine, valued for their capabilities in repelling insects and relieving coughs. The root extracts of these plants can be applied for external use in repelling various insect pests.[11-13] Additionally, *Stemona* root extracts exhibit promising antibacterial properties against some skin infecting pathogens. According to previous research, ethanol extracts of *Stemona tuberosa* roots have been shown to suppress a broad spectrum of bacteria and fungi.[11] More recent studies have isolated eight new alkaloids from the roots of *Stemona tuberosa* and one of these compounds showed good anti-inflammatory activity.[14]

Therefore, to develop an effective herbal product for external use, especially for the skin, an extract of *Stemona tuberosa*, the active component of a traditional formula used for treating skin diseases in Southern Thailand, was tested for its antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl)DPPH(, total phenolic content)TPC(, and ferric-reducing antioxidant power)FRAP(assays. Its toxicity profile using an *in vivo* model, *Galleria mellonella* larvae, was determined, ensuring the safety of the extract for potential cosmetic use, according to the aim of this research. Moreover, consumer acceptance of the resulting body lotion containing various concentrations of *Stemona tuberosa* extracts was assessed, focusing on sensory attributes such as

appearance, fragrance, texture, and functional properties including spreadability and skin nourishment. These results set the stage for a detailed discussion on the viability of *Stemona tuberosa* as a cornerstone ingredient in herbal skincare, its integration into a body lotion, and its implications for the cosmetic industry. It was found to have very good effects as an antioxidant with low toxicity. Extracts of this plant have not been studied before and it appears suitable for inclusion in beauty products.

Materials and Methods

Plant materials

This study utilized various plant materials, including the entire plant, stems, entire plant, bark, and roots of *Rhinacanthus nasutus* (SL001), *Quisqualis indica* (SL002), *Ocimum sanctum* (SL003), *Vitex glabrata* (SL004), and *Stemona tuberosa* (SL005), respectively. These materials were sourced from a certified herbal shop in Songkhla Province, Southern Thailand, at the end of January 2019. It is positioned at 7° 00' 58.0" N latitude and 100° 28' 36.1" E longitude. The medicinal plants employed were adequately authenticated, with their taxonomic identities verified. Voucher specimens were preserved in the Materia Medica herbarium at the Department of Traditional Thai Medicine, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, in Nakhon Si Thammarat, Thailand.

The plant parts were dried in an oven)Binder FD240, Germany(, finely ground (passed through an 80-mesh sieve), individually weighed, and then mixed in precise ratios, as previously outlined. The preparation process, mirroring traditional practices, involved macerating the herbal components in ethanol. Specifically, the mixture was macerated for one week in 70% ethanol at a 1:10 weight/volume ratio, maintained at 30 °C, and continuously agitated at 100 rotations per minute on a shaker)HZ-300, China(. The resultant mixture was then filtered through Whatman No. 1 filter paper. The obtained filtrate was concentrated under reduced pressure at 55 °C using a rotary evaporator)Buchi R-210, Switzerland(. The resulting concentrated polyherbal extracts were then stored at -20 °C and, when needed, dissolved in ethanol to achieve a 25 mg/mL concentration, unless otherwise specified. The yield of the extract from each formulation was calculated based on the initial dry weight of the powdered mix.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

Assessment of the free radical scavenging activity of the extracts was conducted using a mixed-mode mechanism, as outlined in previously published studies focusing on DPPH radicals.[3,15] The efficacy of the standard reference compound, Trolox, and the test extracts were quantified in terms of their 50% inhibitory concentration (IC_{50}) values, expressed in milligrams per milliliter (mg/mL), against radicals. The extracts were prepared in two-fold serial dilutions ranging from 2500 to 1.22 micrograms per milliliter (µg/mL). Twenty microliters (µL) of each dilution were added to individual wells of a 96-well plate containing 180 µL of a DPPH solution at an 80 micromolar (µM) concentration. The mixture was agitated for 5 minutes and then allowed to rest at 25 °C in a dark environment for 30 minutes. The change in the DPPH solution color, indicative of radical scavenging activity, was measured by recording absorbance at 492 nm using a microplate reader (Varioskan Flash, Thermo Fisher Scientific, USA).

Ferric-reducing antioxidant power (FRAP) assay

The capability of the extracts to convert ferric ions in the ferric-tripyridyl triazine (TPTZ) complex to the ferrous form has been previously reported.[3] For this assay, 150 microliters (µL) of each extract, prepared through two-fold serial dilution, were combined with 1.35 milliliters (mL) of freshly prepared FRAP reagent. Following a 2-hour incubation period (in a Binder FD240 incubator, Germany) at 37 °C, the absorbance of the resulting reaction mixture was measured at a wavelength of 596 nm using a microplate reader (Varioskan Flash, Thermo Fisher Scientific, USA). The absorbance readings were then compared to a standard curve generated using ferrous sulfate (Fe_2SO_4) in an ethanol solution. The efficiency of the extracts in reducing ferric ions was quantified in terms of millimoles of ferrous ions per milligram of extract, expressed in micromoles of Fe_2SO_4 per milligram of extract (µM Fe_2SO_4/mg extract).

Total phenolic content (TPC)

A slightly modified Folin-Ciocalteu (FC) method was used to determine the total phenolic content (TPC) of the polyherbal extract as well as the extracts obtained from its herbal components.[3,15] Initially, 120 microliters (µL) of each extract, with a concentration of 2.5 milligrams per milliliter (mg/mL), were combined with 1000 µL of a 10-fold diluted FC reagent and allowed to react for 5 minutes. Following this, 1000 µL of a 20% weight/volume (w/v) sodium carbonate solution (sourced from Ajax Finechem, New Zealand) was added to the mixture. After thorough mixing, the solution was kept under dark conditions at 25 °C for 1.5 hours. The absorbance of the solution was then measured at a wavelength of 725 nm using a Sunrise™ Microplate reader from Tecan Group Ltd., Switzerland. The TPC was expressed as milligrams of gallic acid (obtained from Sigma-Aldrich Chemie, Germany) per gram of the extract.

In vivo toxicity

The acute toxicity of various medicinal plant extracts and the THR01 formulation was assessed using *Galleria mellonella* larvae, following published methodology.[16,17] Initially, stock solutions of the substances were prepared in 100% ethanol, from which serial dilutions were made using sterile deionized water. For the test, groups of fifteen larvae, chosen randomly, were each injected with 10 microliters (µL) of an extract at concentrations of 50, 100, and 200 milligrams per kilogram (mg/kg). Control groups, one receiving phosphate-buffered saline (PBS) and another left unmanipulated, were included in each set of experiments. Post-injection, the larvae were incubated at 37 °C, and their survival rates were recorded every 24 hours for a total duration of 120 hours. A larva was deemed dead if it showed no response to gentle prodding. Each experimental setup was replicated using larvae from distinct batches. The results are presented as the mean ± standard error of the mean (SEM). An extract was classified as non-toxic if the survival rate was 80% or higher at the maximum dosage tested.

Formulation

Preparation of a *Stemona tuberosa* herbal lotion, in concentrations of 1%, 3%, or 5% (by weight) of the extract, involved several steps. The process started with creation of an oil phase. For this, a mixture comprising stearic acid (10% w/w), isopropyl myristate (4% w/w), Span-80 (2.5% w/w), and glyceryl monostearate (2% w/w) was placed in a porcelain dish and melted at 70 °C. In a separate porcelain dish, the chosen concentration of the extract was combined with Tween 80 (5% w/w) and water. This mixture was also heated to 70 °C. Subsequently, the aqueous phase was gradually added to the oil phase, maintaining constant stirring at 70 °C. After combining the phases, the mixture was allowed to cool to room temperature, with continuous stirring. At the end of production, a perfume (0.5%) was incorporated. The final product was then transferred into an appropriate container. The lotion underwent various physical parameter evaluations post-preparation.

Accelerated stability studies for all formulations were conducted by subjecting the samples to a cycle of freezing and thawing. This involved storing the samples at -20 °C for one day, followed by thawing at 37 °C for another day. This freeze-thaw cycle was repeated for a total of three cycles. Throughout stability testing, various parameters were monitored and recorded. These included the homogeneity of the formulation, its viscosity, any observed physical changes, and pH.

Determination of organoleptic properties of Stemona tuberosa herbal lotion

Herbal lotion appearance was assessed by examining its color, pearlescence, and surface texture.[18,19] A calibrated pH meter)Apera, PH700) probe was immersed in a beaker with 20 mg of the lotion for pH measurements. The lotion's uniformity was determined through visual inspection and tactile evaluation. Additionally, aspects of the immediate effects of the lotion on the skin, such as grittiness, viscosity, and overall texture, were examined. In the spreadability assessment, 500 mg of the cream were evenly distributed between two glass slides, and a 100 mg weight was placed atop the upper slide. The lower slide was

attached to a fixed surface after removing the weight and scraping away surplus cream. The upper slide was connected to a non-flexible string bearing a 20 g load. The time required for the upper slide to detach from the lower one was carefully recorded. This procedure was repeated three times, and the mean values of these readings were noted. Spreadability)S(was calculated, as expressed below:

$$S = \frac{m\ x\ l}{t} \qquad (1)$$

where:
m = the weight attached to the upper slide
l = the distance the slide moved
t = the time elapsed until the slide detached

Evaluation of consumer acceptability of a Stemona tuberosa herbal lotion

A panel of 40 semi-trained judges conducted a sensory evaluation of the formulated body lotion samples. This panel carefully assessed the products, focusing on attributes such as color, appearance, fragrance, texture, and overall acceptability.[20,21] A 5-point hedonic scale was employed for a comprehensive and nuanced evaluation. The methodology adopted for determining the consumer acceptability of the *Stemona tuberosa* herbal lotion was thoroughly reviewed and sanctioned by the Human Research Committee at Walailak University, approval number WU-EC-EX-3-015-64.

Statistical analyses

Statistical analysis was done to determine mean values, standard deviation (S.D.) and standard error of the mean (SEM) using Microsoft Office Excel 2016. A paired t-test was used to evaluate antioxidant properties and toxicity. A one-way ANOVA *post-hoc* DUNCAN test was used to evaluate consumer acceptance of herbal lotions with $P < 0.05$ being considered significant. This statistical analysis was performed with SPSS version 21.

Results and Discussion

Table 1 presents data on the extraction yields, *in vitro* antioxidant properties of selected Thai medicinal plants and their formulation, designated as THR-01. The THR-01 formulation was made from five medicinal plants belonging to different families. These plants are *Rhinacanthus nasutus* (Acanthaceae), *Quisqualis indica* (Combretaceae), *Ocimum sanctum* (Lamiaceae), *Vitex glabrata* (Verbenaceae), and *Stemona tuberosa* (Stemonaceae). The parts of the plants used vary from whole plants to specific structures such as stems, bark, and roots. Extraction yields are given as percentages and ranged from 0.15% for *Quisqualis indica* to 2.43% for *Stemona tuberosa*. The combined formulation, THR-01, has a yield of 6.54%.

The concentration of extract required to inhibit 50% of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical activity is a measure of antioxidant capacity. Lower IC_{50} values indicate more potent antioxidant activity. The IC_{50} values ranged from 5.80 µg/mL for *Stemona tuberosa* to 49460.50 µg/mL for *Rhinacanthus nasutus*. The THR-01 formulation has an IC_{50} value of 2025.7 µg/mL, which is lower than that of *Stemona tuberosa* and *Vitex glabrata*.

The total phenolic content, measured in milligrams of gallic acid equivalents per gram of plant material, indicates the level of phenolic compounds that are often associated with antioxidant properties. As expected, the values range from 12.19 mg GAE/g for *Ocimum sanctum* to 869.65 mg GAE/g for *Stemona tuberosa*. THR-01 has a TPC of 19.92 mg GAE/g, lower than that of *Stemona tuberosa*, *Quisqualis indica*, and *Vitex glabrata*.

Ferric reducing antioxidant power, measured in micromoles of ferrous ion (Fe^{2+}) equivalents per gram, is another parameter indicating the antioxidant capacity of test samples. The values ranged from 106.99 µM Fe^{2+}/g for *Rhinacanthus nasutus* to 18108.49 µM Fe^{2+}/g for *Stemona tuberosa*. THR-01 has a FRAP of 184.21 µM Fe^{2+}/g, lower than that of *Stemona tuberosa, Quisqualis indica,* and *Vitex glabrata*.

Among the herbal components of THR-01, *Stemona tuberosa* exhibits the highest yield and the most potent activities in the DPPH, TPC, and FRAP assays. This indicates that it possesses the most potent antioxidant properties which are significant greater ($P < 0.05$) compared to THR-01 and the individual plant components of this standard lotion. This is consistent with observations of numerous Thai herbs, including *Streblus asper*, *Diospyros rhodocalyx*, and *Albizia procera*, which have been investigated for their antioxidant properties. The findings showed that a 50% ethanol extract had elevated levels of total flavonoid content (5.132±0.082 mg QE/g Ext) and TPC (3.617±0.763 mgGE/gExt). Moreover, the IC_{50} values for DPPH (0.0323±0.0008 mg/mL), ABTS (0.0159±0.0004 mg/mL), and FRAP (10.013±0.810 mgTE/gExt) assay are reported. It would be interesting to investigate this further at a clinical level.[22]

The antioxidant properties, toxicity, and consumer appeal of the individual herbal components as well as the combined THR-01 formulation were systematically evaluated. The results demonstrated that *Stemona tuberosa*, an integral component of THR-01, displayed the highest yield among the tested medicinal plants. This is particularly significant as antioxidants offer a range of benefits for skin health and protection. They combat oxidative stress caused by environmental factors such as pollution and UV radiation, which can lead to premature aging, inflammation, and various skin diseases.[23]

Antioxidants neutralize free radicals, which are molecules that can damage skin cells and collagen, leading to wrinkles and fine lines.[24,25] For example, green tea extract is rich in polyphenols including epigallocatechin gallate (EGCG), known for its anti-aging properties. Some herbal antioxidants stimulate skin cell regeneration and repair. Aloe vera contains aloesin and other compounds that can help heal sunburn and reduce skin inflammation.[26] Antioxidants can provide additional protection against the sun's harmful UV rays. Ingredients such as pomegranate extract have compounds that can reduce the damage caused by UVB rays. Herbal extracts can improve skin tone by reducing pigmentation and dark spots.[27] Licorice root extract contains glabridin, which inhibits the enzyme responsible for skin darkening.[28] Many herbal antioxidants also offer moisturizing benefits. For instance, chamomile contains bisabolol, an anti-irritant that also provides moisture, helping to soothe dry or irritated skin.[29] Antioxidant-rich herbs can have anti-inflammatory effects.

Table 1: Extraction yields and *in vitro* antioxidant properties of selected Thai medicinal plants and their formulation

Medicinal plants	Part used	Voucher Numbers	Yield	DPPH (IC_{50}; µg/ml)	TPC (mg GAE/g)	FRAP (µMFe^{+2}/g)
Rhinacanthus nasutus	Whole plant	SL001	0.87	49460.50 ± 155.80	13.54 ± 1.68	106.99 ± 7.14
Quisqualis indica	Stem	SL002	0.15	445.90 ± 60.20	37.86 ± 2.21	681.67 ± 26.01
Ocimum sancium	Whole plant	SL003	0.83	2655.70 ± 112.60	12.19 ± 0.55	107.81 ± 11.37
Vitex glabrata	Bark	SL004	0.68	373.90 ± 50.60	39.41 ± 3.59	585.08 ± 25.90
Stemona tuberosa	Root	SL005	2.43	5.80 ± 0.80	869.65 ± 76.76*	18108.49 ± 84.07*
THR-01	-	-	6.54	2025.7 ± 104.10	19.92 ± 0.64	184.21 ± 5.00

* p-value ≤ 0.05

A positive control, Trolox, possessed a free-radical scavenging effect toward DPPH radicals with an IC_{50} of 16.70 ± 0.40 µg/mL

Turmeric, with its active compound curcumin, is widely recognized for reducing inflammation and redness in skin conditions.[30] Some antioxidants have antimicrobial effects, protecting the skin from bacteria and fungi. Tea tree oil is a notable example. It is often used to treat acne and other skin infections.[31]

Table 2 lists the survival rates of the larvae over time after treatment with various concentrations (50, 100, and 200 mg/kg) of THR-01, and extracts of its herbal components. The data in this table is presented as the percentage of survivors at different times (24, 48, 72, 96, and 120 hours) after treatment. *Rhinacanthus nasutus*, *Quisqualis indica* (except at a 100 mg/kg concentration), *Ocimum sanctum*, and THR-01 (at 50 mg/kg) showed 90% survival after all times. *Vitex glabrata* displayed a decrease in survival rates with increasing concentration. At 50 mg/kg, survival was 93.33%, and this decreased significantly at higher doses)$P < 0.05$) compared with THR-01, with no survivors at the 200 mg/kg dose by 96 hours. It should be noted that *Stemona tuberosa*, a potent antioxidant component of THR-01, generally shows high survival rates, but there was a gradual decline with increasing concentration and time. At 50 mg/kg, the survival rate was 86.67%, and at 200 mg/kg, it decreased to 63.33% by 120 hours, with no significant differences when compared to THR-01 at all concentrations. The consistent 100% survival rate in several groups suggests that the lower concentrations of the individual plants and the THR-01 formulation are non-toxic to the larvae. The data indicates increasing toxicity with higher concentrations for *Vitex glabrata*. Therefore, the *Stemona tuberosa* extract was chosen for development of a plant-based body lotion due to its high antioxidant properties and low toxicity.

Stemona tuberosa, primarily known for its antitussive and insecticidal properties, also possesses several biological activities that may benefit skin health.[17,32,33] The extracts and derived compounds of *Stemona tuberosa* demonstrate antimicrobial and anti-inflammatory activities, which can be beneficial for skin health.[32,34] This property is especially useful in treating or preventing skin infections caused by bacteria or fungi. The anti-inflammatory properties of *Stemona tuberosa* extracts can be beneficial in treating inflammatory skin conditions like acne, eczema, and psoriasis.[33] Interestingly, while *Stemona tuberosa* is a potent antioxidant, it also displayed high survival rates in tests using *Galleria mellonella* larvae, signifying low toxicity at higher concentrations than other components, such as *Vitex glabrata*.

Table 2: Assessing the relative toxicity of selected Thai medicinal plants and their formulation using *Galleria mellonella* larvae as an *in vivo* model (n = 30, mean ± SEM)

Treatment group	**Survivors (%) at each time point (h)**				
	24	**48**	**72**	**96**	**120**
Rhinacanthus nasutus					
50 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
200 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Quisqualis indica					
50 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100 mg/kg	100 ± 0	96.67 ± 0.48	96.67 ± 0.48	96.67 ± 0.48	96.67 ± 0.48
200 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Ocimum sancium					
50 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
100 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
200 mg/kg	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Vitex glabrata					
50 mg/kg	93.33 ± 0.98	93.33 ± 0.98	93.33 ± 0.98	93.33 ± 0.98	93.33 ± 0.98
100 mg/kg	90 ± 0.50	36.67 ± 0.78	36.67 ± 0.78	23.33 ± 2.93	16.67 ± 5.77
200 mg/kg	53.33 ± 2.58	10 ± 4.47	10 ± 4.47	0 ± 0*	0 ± 0*
Stemona tuberosa					
50 mg/kg	86.67 ± 1.01	86.67 ± 1.01	86.67 ± 1.01	86.67 ± 1.01	86.67 ± 1.01
100 mg/kg	86.67 ± 1.01	86.67 ± 1.01	83.33 ± 0.52	76.67 ± 0.54	73.33 ± 1.10
200 mg/kg	83.33 ± 0.52	80 ± 0	76.67 ± 0.54	66.67 ± 0	63.33 ± 0.59
THR-01					
50 mg/kg	90 ± 1.49	90 ± 1.49	90 ± 1.49	90 ± 1.49	90 ± 1.49
100 mg/kg	80 ± 2.11	76.67 ± 2.69	76.67 ± 2.69	76.67 ± 2.69	76.67 ± 2.69
200 mg/kg	86.67 ± 2.03	80 ± 1.05	80 ± 1.05	76.67 ± 1.62	76.67 ± 1.62

Table 2 (continued) Assessing the relative toxicity of selected Thai medicinal plants and their formulation using *Galleria mellonella* larvae as an *in vivo* model (n = 30, mean ± SEM)

Treatment group	**Survivors (%) at each time point (h)**				
	24	**48**	**72**	**96**	**120**
PBS)positive control(	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Unmanipulated	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

* p-value ≤ 0.05

This dual characteristic of high antioxidant capacity and low toxicity makes *Stemona tuberosa* an exceptional candidate for topical applications, hence its selection for developing a plant-based body lotion.

The ingredients used to make herbal lotions with different concentrations of *Stemona tuberosa* extract are described in Table 3. Three formulations are shown as Stu-1, Stu-3, and Stu-5, with the trailing number indicating the percentage of *Stemona tuberosa* extract in the formulation. The common ingredients across all three formulations, with their respective quantities of extract (%w/w) were stearic acid, isopropyl myristate, glyceryl monostearate, Span-80, methylparaben, Tween 80, and natural fragrance. These ingredients serve as the lotion base, while varying amounts of *Stemona tuberosa* extract are tested for efficacy. The *Stemona tuberosa* extract was varied in concentrations of 1%, 3%, and 5% w/v for the Stu-1, Stu-3, and Stu-5 formulations, respectively.

The choice of ingredients aimed to establish a stable base that enables variation of the concentration of *Stemona tuberosa* extract to evaluate its efficacy. Common ingredients such as stearic acid and glyceryl monostearate ensure a consistent texture and moisturizing properties across all formulations. An investigation into the texture and stability of these lotions showed that all formulations maintained their homogeneity and smoothness post-freeze-thaw testing, an encouraging sign for the shelf-life of the product.

Data in Table 4 provides a comprehensive overview of the lotion properties, indicating their texture, stability, and suitability for use both when freshly prepared and after undergoing stress testing, which simulates potential changes during storage and use. Two lotions, freshly prepared product (FPP) and after freeze-thaw (FT), are tested for each formulation. For their homogeneity and grittiness, all tested products are noted as smooth, both as FPP and after FT testing with no grittiness reported for any products under either condition. According to their visual appearance, all freshly prepared products (FPP) are described as white/creamy. After freeze-thaw testing, the Stu-1 and Stu-3 products maintain a white/creamy appearance, while Stu-5 becomes yellowish/softer. All FPPs have the highest viscosity rating (+++). After FT testing, Stu-1 and Stu-3 maintain this viscosity, while Stu-5 decreases slightly (++). Spreadability is described as good for Stu-1 FPP and Stu-5 FPP and FT, and very good for Stu-3 FPP and Stu-1 FT. The FPP samples at all concentrations have a light texture. After FT, Stu-1 and Stu-3 maintain a light texture, but Stu-5 becomes sticky. The pH values range from 4.8 for Stu-1 FPP and Stu-5 FPP, to 6.2 for Stu-3 FT. No microbial growth was observed in any of the products under either condition. However, a notable shift was observed in the viscosity and spreadability post-testing, especially for Stu-5. This formulation exhibited a change in visual appearance to a yellowish color, softer consistency, and a sticky texture. These changes highlight the importance of considering the effects of storage conditions and product stability over time.[35,36]

Table 5 presents the results of consumer acceptance testing of herbal lotions with three different concentrations of *Stemona tuberos*a extract (Stu-1, Stu-3, Stu-5). The appearance scores ranged from 3.62 for Stu-1 to 3.75 for Stu-3, indicating moderate to high acceptance, with Stu-3 rated marginally higher. In terms of fragrance, Stu-3 received the highest score, 3.63, suggesting a more favorable scent than Stu-1, 3.27. Stu-5 had a fragrance score similar to Stu-3. All formulations scored above 4 for color, demonstrating a high level of consumer acceptance, and Stu-3 led slightly with a score of 4.10. Texture scores increased with the concentration of extract, from 3.00 for Stu-1 to 3.40 for Stu-5. The spreadability of the lotions was rated as good for all formulations, with Stu-5 receiving the highest score, 3.62. Stu-3 and Stu-5 scored 3.92 for skin nourishment, higher than the 3.60 score for Stu-1, indicating a perception of better nourishment with higher extract concentrations. The overall acceptability was high for all lotions. Stu-3 achieved the highest score, 4.02, followed closely by Stu-5. The results shown by Stu-3 and Stu-5, however, indicate that consumer acceptance is trending in the same direction and that there is little variation, while Stu-1 has significantly lower scores compared to the two previous formulae.

Consumer acceptance testing revealed high scores for appearance, fragrance, and color, which are essential for market success. The lotion texture and spreadability were also rated positively, with higher extract concentrations correlating with increased consumer satisfaction, especially regarding skin nourishment.[37,38] Overall, Stu-3 and Stu-5 were favored, suggesting that a moderate to high concentration of *Stemona tuberosa* extract may be optimal to satisfy consumer preferences. Currently, there is global interest in creating of cosmetics with active components derived from natural extracts. Addition of coffee extract showed high physical stability and considerably improved antioxidant activities at concentrations of 0.25%, 0.25%, 0.5%, and 1% by mass. Additionally, customer opinions of products made with natural substances are also highly positive, similar to this study.[39,40] Furthermore, without the trouble of processing several herbs at once, this research demonstrates that *Stemona tuberosa*, a single medicinal plant, has significant potential as an ingredient in lotions.

In summary, incorporating herbal-based antioxidants into body lotions not only enhances the product's capability to protect and repair skin, but also provides a natural and holistic approach to skincare. With growing consumer awareness of the importance of natural ingredients, these antioxidants can increase a product's appeal in the skincare market.

Table 3: Ingredients of herbal lotions containing different concentrations of *Stemona tuberosa* extract

Ingredients	**Quantity (%w/w)**		
	Stu-1	**Stu-3**	**Stu-5**
Stemona tuberosa extract	1	3	5
Stearic acid	10	10	10
Isopropyl myristate	4	4	4
Glyceryl monostearate	2	2	2
Span-80	2.5	2.5	2.5
Methyl paraben	1.75	1.75	1.75
Tween 80	5	5	5
Natural fragrance	0.5	0.5	0.5
Water	73.25 q.s.	71.25 q.s.	69.25 q.s.

q.s. (Quantity sufficient)

Table 4: Texture and stability of herbal lotions containing different concentrations of *Stemona tuberosa* extract

Tested property	Product tested					
	Stu-1		Stu-3		Stu-5	
	FPP	FT	FPP	FT	FPP	FT
Visual	White/ Creamy	White/ Creamy	White/ Creamy	White/ Softer	White/ Creamy	Yellowish/ Softer
Homogeneity	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Grittiness	-	-	-	-	-	-
Viscosity	+++	+++	+++	+++	+++	++
Spreadability	Good	Very good	Good	Very good	Good	Greasy
Texture	Light	Light	Light	Light	Light	Sticky
pH	4.8	5.5	4.8	6.2	4.8	5.0
Test for microbial growth	No growth	No growth	No growth	No growth	No growth	No growth

#FPP; Freshly prepared product: FT; after three cycles of freeze-thaw testin

Table 5: Consumer acceptance of herbal lotions containing different concentrations of *Stemona tuberosa* extract

Tested property	Product tested		
	Stu-1	Stu-3	Stu-5
Appearance	3.62 ± 0.87^{a}	3.75 ± 0.70^{a}	3.70 ± 0.75^{a}
Fragrance/Smell	3.27 ± 0.78^{b}	3.63 ± 0.90^{a}	3.62 ± 0.97^{a}
Color	4.05 ± 0.87^{a}	4.10 ± 0.88^{a}	4.07 ± 0.85^{a}
Texture	3.00 ± 1.08^{a}	3.17 ± 1.10^{a}	3.40 ± 0.90^{a}
Spreadability	3.55 ± 0.95^{a}	3.27 ± 0.93^{b}	3.62 ± 0.83^{a}
Skin nourishment	3.60 ± 0.87^{b}	3.92 ± 0.65^{a}	3.92 ± 0.76^{a}
Overall acceptability	3.7 ± 0.68^{b}	4.02 ± 0.58^{a}	3.9 ± 0.81^{a}

Means in the same row that are followed by a different superscript letter differ significantly ($p < 0.05$)

Conclusion

The study findings suggest that *Stemona tuberosa* is well-suited for use in a body lotion formulation due to its potent antioxidant activity and low toxicity. Consumer acceptance testing further indicates that the product has the potential to be well-received in the market, especially if the formulations are optimized based on the observed effects of concentration on the product's physical properties and consumer preferences. In the future, the effectiveness of the product should be tested using volunteers, assessing aspects such as melanin pigment dispersion, hydration and skin firmness. As a confirmation, the product can be marketed.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Chokpaisarn J, Yincharoen K, Sanpinit S, Karutha Pandian ST, Nandhini JR, Gowrishankar S, Limsuwan S, Kunworarath N, Voravuthikunchai SP, Chusri S. Effects of a traditional Thai polyherbal medicine 'Ya-Samarn-Phlae' as a natural anti-biofilm agent against *Pseudomonas aeruginosa*. Microb Pathog. 2019; 128:354-362. Doi: 10.1016/j.micpath. 2019.01.036
2. Chumpolphant S, Suwatronnakorn M, Issaravanich S, Tencomnao T, Prasansuklab A. Polyherbal formulation exerts wound healing, anti-inflammatory, angiogenic and antimicrobial properties: Potential role in the treatment of diabetic foot ulcers. Saudi J Biol Sci. 2022; 29(7):103330. Doi: 10.1016/j.sjbs.2022.103330
3. Lohakul J, Chaiprasongsuk A, Jeayeng S, Saelim M, Muanjumpon P, Thanachaiphiwat S. Panich U. The protective effect of polyherbal formulation, harak formula, on uva-induced photoaging of human dermal fibroblasts and mouse skin via promoting nrf2-regulated antioxidant defense. Frontiers in pharmacology. 2021; 12: 649820. Doi: org/10.3389/fphar.2021.649820
4. Shen T, Xing G, Zhu J, Cai Y, Zhang S, Xu G, Feng Y, Li D, Rao J, Shi R. Effects of 12-week supplementation of a polyherbal formulation in old adults with prehypertension/hypertension: a randomized, double-blind, placebo-controlled trial. Evid Based Complement Alternat Med. 2019; 2019:7056872. Doi.org/10.1155/2019/7056872
5. Dubey S, Dixit AK. Preclinical evidence of polyherbal formulations on wound healing: A systematic review on research trends and perspectives. J Ayurveda Integr Med. 2023; 14(2):100688. Doi: 10.1016/j.jaim.2023.100688
6. Srivastava A, Rengaraju M, Srivastava S, Narayanan V, Gupta V, Upadhayay R, Kumar J, Parameswaran S, Kadarkarai K, Velmurugan A. Efficacy of two siddha polyherbal decoctions, Nilavembu Kudineer and Kaba Sura Kudineer, along with standard allopathy treatment in the management of mild to moderate symptomatic COVID-19 patients-a double-blind, placebo-controlled, clinical trial. Trials. 2021; 22(1):570. Doi: 10.1186/s13063-021-05478-0
7. Siddique YH. Role of Unani polyherbal formulations in the treatment of diseases with special reference to neurodegenerative disorders. CNS Neurol Disord Drug Targets. 2023; 22(3):321-328. Doi: 10.2174/1871527321666220127141611
8. Van Vuuren SF, Motlhatlego KE, Netshia V. Traditionally used polyherbals in a southern African therapeutic context. J Ethnopharmacol. 2022; 288:114977. Doi: 10.1016/j.jep.2022.114977
9. Karole S, Shrivastava S, Thomas S, Soni B, Khan SU, Dubey J, Dubey SP, Khan N, Jain DK. Polyherbal formulation concept for synergic action: a review. J Drug Deliv Ther. 2019; 9(1-s):449-452. Doi:10.22270/jddt.v9i1-s.2339
10. Saising J, Maneenoon K, Sakulkeo O, Limsuwan S, Gotz F, Voravuthikunchai S P. Ethnomedicinal Plants in Herbal Remedies Used for Treatment of Skin Diseases by Traditional Healers in Songkhla Province, Thailand. Plants. 2022; 11(7): 880. Doi: org/10.3390/plants11070880

11. Liu Y, Shen Y, Teng L, Yang L, Cao K, Fu Q, Zhang J. The traditional uses, phytochemistry, and pharmacology of Stemona species: A review. J Ethnopharmacol. 2021; 265:113112. Doi: 10.1016/j.jep.2020.113112
12. Loahaprapanon S, Yincharoen K, Nukong J, Chanwun T. An Investigation on the Antibacterial and Antibiofilm Efficacy of a Traditional Thai Herbal Recipe (THR 01) against Clinical Isolates of *Methicillin Resistant Staphylococcus Aureus* (MRSA) and *Staphylococcus epidermidis*. International Journal of Agricultural Technology. 2018; 14(3): 325-332.
13. Sakulpanich A, Attrapadung S, Gritsanapan W. Larvicidal activity of *Stemona collinsiae* root extract against *Musca domestica* and *Chrysomya megacephala*. Sci Rep. 2023; 13(1):15689. Doi.org/10.1038/s41598-023-42500-8
14. Xu Y, Xiong L, Yan Y, Sun D, Duan Y, Li H, Chen L. Alkaloids from *Stemona tuberosa* and their anti-inflammatory activity. Frontiers in Chemistry. 2022; 10:847595. Doi.org/10.3389/fchem.2022.847595
15. Ruangchuay S, Wang QQ, Wang LY, Lin J, Wang YC, Zhong GH, Maneenoon K, Huang ZB, Chusri S. Antioxidant and antiaging effect of traditional Thai rejuvenation medicines in *Caenorhabditis elegans*. J Integr Med. 2021; 19(4):362-373. Doi.org/10.1016/j.joim.2021.03.004
16. Siriyong T, Voravuthikunchai SP, Coote PJ. Steroidal alkaloids and conessine from the medicinal plant Holarrhena antidysenterica restore antibiotic efficacy in a Galleria mellonella model of multidrug-resistant *Pseudomonas aeruginosa* infection. BMC Complementary and Altern Med. 2018; 18(1):1-10. Doi.org/10.1186/s.12906-018-2348-9
17. Mbarga MJA, Podoprigora IV, Anyutoulou K LD. *Galleria mellonella* (greater wax moth) as an eco-friendly in vivo approach for the assessment of the acute toxicity of medicinal plants: Application to some plants from Cameroon. Open Vet. J. 2021; 11(4): 651-661. Doi: org/10.5455/OVJ.2021.v11.i4.15
18. Ilomuanya M O, Ekerebe Z, Cardoso-Daodu I, Sowemimo A. Formulation and Evaluation of Sunscreen Cream Using Detarium senegalenseOil as Base. Trop J Nat Prod Res. 2020; 4(4):141-145. Doi.org/10.26538/tjnpr/v4i4.5
19. Rasidah R, Maurizka I S, Aulianshah V, Munira M, Zakiah N, Handayani R. Repellent Activity of Lotion of Essential Oils from *Piper betle* Linn and *Cymbopogon nardus* [L.] Trop J Nat Prod Res. 2022; 6(12):2020-2024. Doi.org/10.26538/tjnpr/v6i12.20
20. Belmes NJ. BATUAN (Garciniabinucao) fruit preservation. In Journal of Physics: Conference Series. 2019; 1254)1(: 012030. Doi: 10.1088/1742-6596/1254/1/012030
21. Tan PL, Rajagopal M, Chinnappan S, Selvaraja M, Leong MY, Tan LF, Yap VL. Formulation and physicochemical evaluation of green cosmeceutical herbal face cream containing standardized mangosteen peel extract. Cosmetics. 2022; 9(3):46. Doi: org/10.3390/cosmetics9030046
22. Konsue A, Taepongsorat L. Phytochemical Screening and Antioxidant Activity of Longevity Remedy from National Thai Traditional Medicine Scripture (Formulary Special Edition). Trop J of Nat Prod Res. 2022; 6(6). Doi.org/10.26538/tjnpr/v1i4.5
23. Chen J, Liu Y, Zhao Z, Qiu J. Oxidative stress in the skin: Impact and related protection. Int J Cosmet Sci. 2021; 43(5):495-509. Doi: 10.1111/ics.12728
24. Azevedo Martins TE, Sales de Oliveira Pinto C A, Costa de Oliveira A, Robles Velasco MV, Gorriti Guitierrez AR, Cosquillo Rafael M F, Retuerto-Figueroa MG. Contribution of topical antioxidants to maintain healthy skin-A review. Scientia Pharmaceutica. 2020; 88(2): 27. Doi.org/10.3390/scipharm88020027
25. Michalak M. Plant-derived antioxidants: Significance in skin health and the ageing process. International journal of molecular sciences. 2022; 23(2): 585. Doi: org/10.3390/ijms23020585
26. Kim E, Hwang K, Lee J, Han SY, Kim EM, Park J, Cho JY. Skin protective effect of epigallocatechin gallate. Int J Mol Sci. 2018; 19(1):173. Doi: 10.3390/ijms19010173
27. Cerulli A, Masullo M, Montoro P, Piacente S. Licorice (*Glycyrrhiza glabra, G. uralensis*, and *G. inflata*) and their constituents as active cosmeceutical ingredients. Cosmetics. 2022; 9(1): 7. Doi: org/10.3390/cosmetics9010007
28. Henning SM, Yang J, Lee RP, Huang J, Hsu M, Thames G, Gilbuena I, Long J, Xu Y, Park EH, Tseng CH, Kim J, Heber D, Li Z. Pomegranate juice and extract consumption increases the resistance to uvb-induced erythema and changes the skin microbiome in healthy women: a randomized controlled trial. Sci Rep. 2019; 9(1):14528. Doi: 10.1038/s41598-019-50926-2
29. Sah A, Naseef PP, Kuruniyan MS, Jain GK, Zakir F, Aggarwal G. A Comprehensive study of therapeutic applications of chamomile. Pharmaceuticals (Basel). 2022; 15(10):1284. Doi: 10.3390/ph15101284
30. Kumar B, Aggarwal R, Prakash U, Sahoo PK. Emerging therapeutic potential of curcumin in the management of dermatological diseases: an extensive review of drug and pharmacological activities. Future J Pharm Sci. 2023; 9(1):42. Doi.org/10.1186/s43094-023-00493-1
31. Michalak M. Plant-derived antioxidants: Significance in skin health and the ageing process. Int J Mol Sci. 2022; 23(2):585. Doi: 10.3390/ijms23020585
32. Liu Y, Shen Y, Teng L, Yang L, Cao K, Fu Q, Zhang J. The traditional uses, phytochemistry, and pharmacology of Stemona species: A review. Journal of Ethnopharmacology.2021; 265: 113112. Doi: org/10.1016/j.jep.2020.113112
33. Greger H. Structural classification and biological activities of Stemona alkaloids. Phytochemistry Reviews. 2019; 18(2): 463-493. Doi: org/10.1007/s11101-019-09602-6
34. Liu Y, Shen Y, Teng L, Yang L, Cao K, Fu Q, Zhang J. The traditional uses, phytochemistry, and pharmacology of Stemona species: A review. J Ethnopharmacol. 2021; 265:113112. Doi: 10.1016/j.jep.2020.113112
35. Priyadarsini SS. Formulation and evaluation of a herbal antibacterial cream from ethyl acetate extract of leaves of *Spinacia oleracea* Linn. against *Aeromonas* skin and soft tissue infections. Int J Green Pharm. 2018; 12(3):S537-S542. Doi.org/10.22377/ijgp.v12i03.2015
36. Gupta N, Dubey A, Prasad P, Roy A. Formulation and evaluation of herbal fairness cream comprising hydroalcoholic extracts of *Pleurotus ostreatus, Glycyrrhiza glabra* and *Camellia sinensis*. J Pharm Biosci. 2015; 3(3):40-45. Doi:10.20510/ukjpb/3/i3/89410
37. Soto ML, Parada M, Falqué E, Domínguez H. Personal-care products formulated with natural antioxidant extracts. Cosmetics. 2018; 5(1):13. Doi.org/10.3390/cosmetics5010013
38. Rodrigues F, Sarmento B, Amaral MH, Oliveira MBPP. Exploring the antioxidant potentiality of two food by-products into a topical cream: stability, *in vitro* and *in vivo* evaluation. Drug Dev Ind Pharm. 2016; 42(6):880-889. Doi: 10.3109/03639045.2015.1088865
39. Widiputri D I, Wijaya S, Kusumocahyo SP. Development of skin lotion containing antioxidant extract from coffee pulp and study on its stability. In IOP Conference Series: Materials Science and Engineering.2020; 742)1(: 012020. Doi: 10.1088/1757-899X/742/1/012020
40. Kusumastuti A, Rahma HS. Application of parijoto (*Medinilla speciosa* l.,) extract as body lotion. In IOP Conference Series: Earth and Environ Sci. 2021; 810)1(: 012054. Doi: 10.1088/1755-1315/810/1/012054.