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Research Article

Formulation And Evaluation Of Herbal Cream For The Treatment Of Wound Healing

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ABSTRACT

This study was to formulate and evaluate an herbal cream for wound healing using natural plant extracts known for their therapeutic properties. The selected herbs included Centella asiatica, which was chosen based on their well-documented efficacy in promoting skin repair and regeneration. Herbal extracts were prepared using ethanol and water as solvents, followed by standardization to ensure consistent active compound concentrations. The cream base was developed as an oil-in-water emulsion, optimized for pH, viscosity, and Spreadability to ensure suitability for topical application. Comprehensive stability studies were conducted to assess the formulation's physical, chemical, and microbial stability. The wound healing efficacy was evaluated through in vitro scratch assays and in vivo studies using a rat model, demonstrating significant improvements in wound contraction and re-epithelialization compared to controls. Additionally, dermal irritation tests confirmed the formulation's safety for skin application. This research highlights the potential of combining traditional herbal medicine with modern formulation techniques to create effective and safe wound-healing products. The findings suggest that the developed herbal cream is a promising alternative to conventional treatments, offering natural and accessible solutions for wound care.

Keywords: Herbal cream, Wound healing, Centella asiatica, Topical formulation.

INTRODUCTION:

Cream:

Creams are semi-solid emulsions used on the skin for therapeutic, protective, or cosmetic purposes. They combine water and oil phases, stabilized by emulsifiers, to form a smooth mixture. Key components include water for hydration, oils for moisturizing, emulsifiers for stability, thickeners for consistency, humectants for moisture retention, preservatives for safety, and active ingredients for specific benefits

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Creams come in two varieties: water-in-oil (richer texture) or oil-in-water (lighter texture).. They deliver active ingredients like anti-inflammatories or vitamins, enhancing skin health and appearance.

They are popular due to their ease of application, pleasant feel, and effectiveness in treating various skin conditions. Herbal creams, made with plant-derived ingredients, are increasingly favored for their natural benefits. (1)

Natural and Herbal Cream:

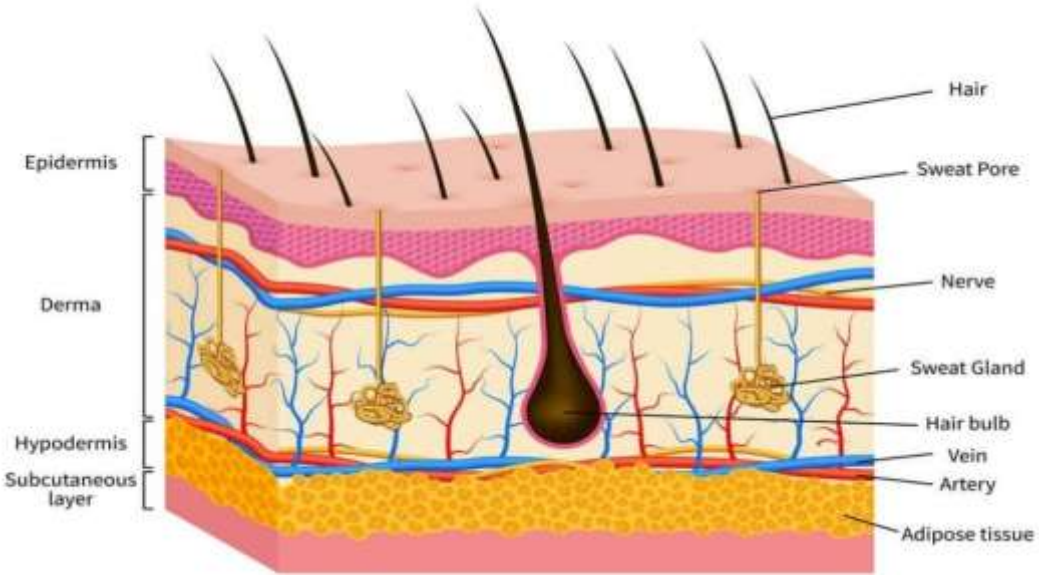
The increasing demand for natural and organic products has led to the development of herbal creams.

These creams use plant-derived ingredients and botanical extracts, offering holistic skincare solutions with perceived safety and minimal environmental impact. Herbal creams combine traditional medicinal knowledge with modern scientific research to provide effective natural therapies.

Human Skin:

Human skin is a complex and dynamic organ that serves several essential functions. Here's an overview of the structure and functions of human skin. (2)

Figure No.1. Anatomy of Human skin



Epidermis:

The outermost layer of the skin is primarily composed of keratinocytes. Provides a protective barrier against environmental factors.

Dermis:

Lies beneath the epidermis and contains connective tissue, blood vessels, nerves, and appendages like hair follicles and sweat glands. Contributes to the skin's strength, elasticity, and resilience.

Hypodermis (Subcutaneous Tissue):

Composed of adipose (fat) tissue and connective tissue. Provides insulation, cushioning, and energy storage.

Functions of the Skin:

I. Barrier Function:

Protects the body from pathogens, chemicals, and physical trauma.

II. Thermoregulation:

Regulates body temperature through processes like sweating and vasodilation/vasoconstriction.

III. Sensation:

Houses sensory receptors for touch, temperature, and pain perception.

IV. Synthesis of Vitamin D:

Converts sunlight into vitamin D, essential for calcium metabolism.

V. Immune Response:

Contains immune cells (e.g., Langerhans cells) that participate in immune responses.

VI. Excretion:

Eliminates waste products through sweat, contributing to detoxification.

Wound and Wound healing:

A wound is the disruption or loss of cellular, anatomical, or functional integrity in living tissue, typically resulting from physical trauma, chemical exposure, heat, microbial invasion, or immunological reactions. The body possesses an inherent wound-healing capacity, involving the regeneration of cutaneous and dermal tissues. This process requires collaboration among various tissue types and cell lineages to facilitate healing. Key components include angiogenesis, the formation of new blood vessels; re-epithelialization, the renewal of epithelial cells to cover the wound; alterations in the extracellular matrix; blood coagulation to stop bleeding; platelet aggregation; fibrin formation; and inflammatory response. These intricate mechanisms promote effective wound healing and restore tissue integrity. (3)

Centella Asiatica Plant:

Centella Asiatica (CA) is a perennial herbaceous creeper that is clonal and grows up to 1800 meters in height in the damp climate of India. As a member of the Umbellifer (Apiceae) family, it inhabits swampy locales across a spectrum of tropical and subtropical nations, including portions of Madagascar, South Africa, South America, Pakistan, India, and Eastern Europe. Approximately 20 species kin to Centella Asiatica thrive in tropical or moist pantropical regions encompassing rice fields and elevated rocky terrains. This water-adjacent plant bears diminutive oval fruits and delicate fan-shaped green leaves, while its blossoms range from white to light purple or pink, or a fusion of these hues. Remarkably, Centella Asiatica is devoid of both taste and scent.[4-5]

The principal active constituents within Centella asiatica consist of saponins, alternatively termed triterpenoids. Among these are madecassoside, metastatic acid, and Asiaticosides, which constitute trisaccharide–linked aglycones of Asiatic acid and madecassoside.[6]

These triterpenes saponins and their sapogenins facilitate vascular effects and wound healing by impending collagen synthesis at the wound site. Clinical exploration is warranted to ascertain whether additional components extracted from Centella asiatica, such as brahmoside and brahminoside, contribute to CNS and uterorelaxant effects. Antifertility effects were observed in mice treated with a crude extract containing the glycosides thankuniside and isothankuniside.[7-8]

Centelloside and its derivatives have demonstrated efficacy in treating venous hypertension furthermore, the integral extract necessitates further investigation. [9]

Material and Methods:

Material:

The plants were selected based on their antimicrobial activities and their medicinal uses reported in the literature. The herbs Centella asiatica powder were purchased from Shree Ram Online, Odisha, India 767001 by online source, and Active marker Asiaticosides were obtained from Shree Ram Online, Odisha, India 767001 by online source, and Boric acid, Cetyl alcohol, Glyceryl monosterate, Sodium benzoate, Beeswax, Petroleum jelly, Vitamin E and Rose oil were provided by Dev Bhoomi Uttarakhand University, Dehradun, pin code – 248007 and Sesame oil purchased from Reliance super mart Dehradun 248007.

Table No.1. List of Chemical used.

S.No	Name of the chemicals	Manufacturers
1	Centella asiatica powder	Green Earth Products Private Limited New Delhi, India
2	Sesame oil	Earth Science Ayurveda, Faridabad, India
3	Boric acid	Thermo Fisher Scientific India Private Limited, Mumbai
4	Cetyl alcohol	Central Drug House Private Limited, New Delhi
5	Petroleum jelly	Sisco-chemical Private Limited Mumbai
6	Beeswax	Central Drug House Private Limited, New Delhi
7	Glyceryl monostearate	Central Drug House Private Limited, New Delhi
8	Sodium benzoate	Nice Chemical Private Limited Kochi Kerala, India
9	Vitamin E	Nexlife Nutrascience, Ahmedabad
10	Rose oil	Pitambari Products Private Limited Thane, India
11	Distilled water	Saj Water Treat India Private Limited Mumbai

Formulation and Development of Herbal cream:

Pre-formulation study:-

To check for any incompatibilities, infrared (IR) investigations were conducted on the formulation excipients, including sesame oil, boric acid, cetyl alcohol, glyceryl monostearate, and sodium benzoate, using an aqueous extract of Centella Asiatica. These samples were kept at 40 °C for two weeks before being used in a spectrophotometer set between 300 and 3000 cm-1 for FTIR analysis.

Solubility of Aqueous Extract in Oil:

It was ascertained whether the aqueous extract was soluble in oils, namely in sesame, corn, Arachis, and linseed oils. An orbital shaker (model number: CIS-24 BL) was used to store the extract (1 g) in 2 mL for the entire night. The oil was then filtered and subjected to HPTLC analysis.

Selecting a cream formula:

The cream recipe was chosen from existing literature.[10] The composition and amount of materials used to make the cream for treating wounds.

Table No.2. Formula of wound healing herbal cream**.**

Sr.no	**Ingredients**	**T1** %w/w in gram	**T2** %w/w in gram	**T3** %w/w in gram	T4 %w/w in gram	**T5** %w/w in gram	**T6** %w/w in gram	**T7** %w/w in gram
1	Centella asiatica powder	10	10	10	10	10	10	10
2	Sesame oil	20	20	20	20	20	20	20
3	Boric acid	3	3	3	3	3	3	3
4	Cetyl alcohol	10	9	8	7	6	5	5
5	Petroleum jelly	5	5	6	7	7	6	7
6	Beeswax	5	5	4	4	4	3	3
7	Glyceryl monosterate	2	2	2	2	2	2	2
8	Sodium benzoate	1	1	1	2	2	2	2
9	Vitamin E	1	1	1	1	1	1	1
10	Rose oil	0.5	0.5	0.5	1	1	1	1
11	Distilled water	42.5	43.5	44.5	43	44	47	46
12	Total weight of cream	100	100	100	100	100	100	100

Ksheerapaka extraction method:-

The medication, milk, and water are combined in a ratio of 1:8:32 as per the normal preparation protocol. The combination is then cooked until the milk solids separate and the drug's properties are removed. After that, it is administered and strained. It is advised that the boiling procedure be done on a medium flame of heat to extract the best qualities from the milk, even though this is not stated in the literature.[11]

SOXHLET EXTRACTION METHODS:-

The thimble with 100 g of powdered Centella Asiatica was inserted inside the soxhlet chamber. The distillation procedure started after 500 ml of chosen solvents were constructed for the soxhlet extractor and put in a round-bottom flask. The solvent was evaporated by submerging the extractor and solvent in water once the extraction procedure was finished. Next, the extracted extract of Centella Asiatica was weighed using the equation that follows.

$$\% \text{ extract yield} = \frac{w1-w2}{w1} \times 100$$

w1= Where: sample weight initially placed in the thimble and
W2 is the sample weight following oven drying (Natarajan et al. 2003). We calculated the acid value and saponification value using the guidelines provided by the AOAC (1984). The specific gravity and pH were measured using the AOAC (1990) technique.

Extraction of Centella Asiatica by using the Ksheerapaka extraction method:

- Take 10% water and 10% milk and the remaining amount will be Sesame oil which is used as a solvent.
- Take Centella asiatica dried leaves and 5% water and then boil them.
- Add remaining 5%milk heat under continuous stirring.
- Add remaining 80%sesame oil and heat under continuous stirring for 20 minutes.
- Then cool the solution and filter through filter paper.
- The extract of Centella Asiatica the desired consistency was obtained.

Making an herbal cream to heal wounds:

The creams were made utilizing formulas and the fusing process. To create a homogenous liquid, the oil phase ingredients (extract, sesame oil, boric acid, cetyl alcohol, petroleum jelly, beeswax, Glyceryl monostearate, and vitamin E) were weighed and continuously stirred with a mechanical stirrer set at 100 rpm and 75°C. Similarly, a mechanical stirrer operating at 100 rpm and 75°C was used to weigh and continuously stir the water phase constituents (distilled water and sodium benzoate).

Using a mechanical stirrer set at 100 rpm (Remi motor RQT-127 HP1/8) and 0.5 grams of rose oil, the phases were continuously stirred for 30 minutes, or until the ingredients were evenly distributed. Each of these batches was given a full day to acclimate.

Evaluation of herbal wound healing cream:

PH determination:

To prepare 1% w/v concentration, a precisely weighed quantity of cream was distributed in water. To find the pH, a calibrated meter (Make: I, DELUXE-101) was utilized.

Hardness:

Using a texture analyzer (Brookfield CT-3), the hardness of the formulation was assessed. 20 grams of cream were put into a conical probe until it reached the top plane surface. This device compares adhesive force and Spreadibility while displaying hardness.

Viscosity

Density With a viscometer (Brookfield digital viscometer RVDV Pro) fitted with a ULE adaptor, the viscosity of the produced creams was measured. At 0.5 rpm, the spindle (S06) was revolved. Before taking measurements, cream samples were allowed to settle at a temperature of 25±10C for 30 minutes. The reported viscosity was in (cp).[12-13]

Calculating the average diameter of globules [14]

Using a 1% cream dispersion in water, optical microscopy was used to calculate the mean globule diameter.

In-vitro drug release

The drug release from the cream was measured over six hours using the Franz diffusion cell instrument. The receptor medium, phosphate buffer pH 6.8, was utilized and maintained at 370°C. The cellulose acetate membrane filter (pore size 0.45µ) was placed between the donor and receptor compartments after being submerged in phosphate buffer pH 6.8 for an hour. The gel was placed over the receptor compartment, and then the two chambers were secured together using a clamp. The phosphate buffer pH 6.8 in the receptor compartment (8 ml) was stirred with a magnetic stirrer set at 60 rpm. The 1 ml samples were taken out and replaced with an equivalent volume of buffer at different times. The materials were subjected to spectrophotometric analysis at 297 nm. The proportion of all drug releases.[15]

TLC identification:

Cetyl acetate, methanol, and water (10: 2.5: 1, v/v/v) were used as the mobile phase, and precoated silica gel 60 F254 aluminum plates as the stationary phase in the TLC analysis. Anisaldehyde sulfuric reagent was used for spot detection, and it was heated to 100 C for five to ten minutes. Before every analysis, the sample solutions were made from scratch. In triplicate, the ensuing solutions were made.

Sample solution:

Using an ultrasonic bath at 45°C for 10 minutes, a cream solution in 100 mg/ml of methanol was created. This mixture was filtered and allowed to cool to room temperature.

Solution of Centella asiatica extract:

An ultrasonic bath was used to make a solution of the dry extract raw material from Centella asiatica in 1 mg/ml of methanol (45 °C, 10 minutes).

Asiaticosides reference solution:

Asiaticosides were dissolved in methanol at a concentration of 1 mg/ml.

Excipients blank solution:

The preparation process was identical to that of the sample solution.
Applying a 10 mm band with a capillary tube to a TLC plate was the sample solution, Centella asiatica extract solution, Asiaticosides solution, and excipient blank solution. 9.0 centimeters was the development distance.

Result and Discussion:

Creation and composition of cream:

Pre-formulation studies:

Asiaticosides facilitate the development of extracellular matrix and fibroblast growth during wound healing.

In addition to generating pollution, the usage of synthetic and semi-synthetic excipients in cosmetics has long-term adverse effects. With its well-documented natural component and surface-active properties, Centella asiatica extract has been utilized traditionally

One major drawback of using surfactants such as sodium lauryl sulfate (SLS) is that they can easily dissolve the natural oil on the skin in addition to sebum and debris. Skin thickness, irritation, inflammation, and other immune system activities can all be increased by a 2% SLS solution.

In addition to causing redness and swelling, SLS can raise the levels of enzymes in the skin. .. The current study aims to determine whether dried aqueous Centella asiatica extract is suitable as an emulsifier for skin creams.

This would reduce environmental pollution because it is biodegradable and would produce skin-friendly, biocompatible cosmetic preparations.

Finding the aqueous extract's solubility in oil:

The extract soluble fraction that was highest in corn oil is shown in the figure.2

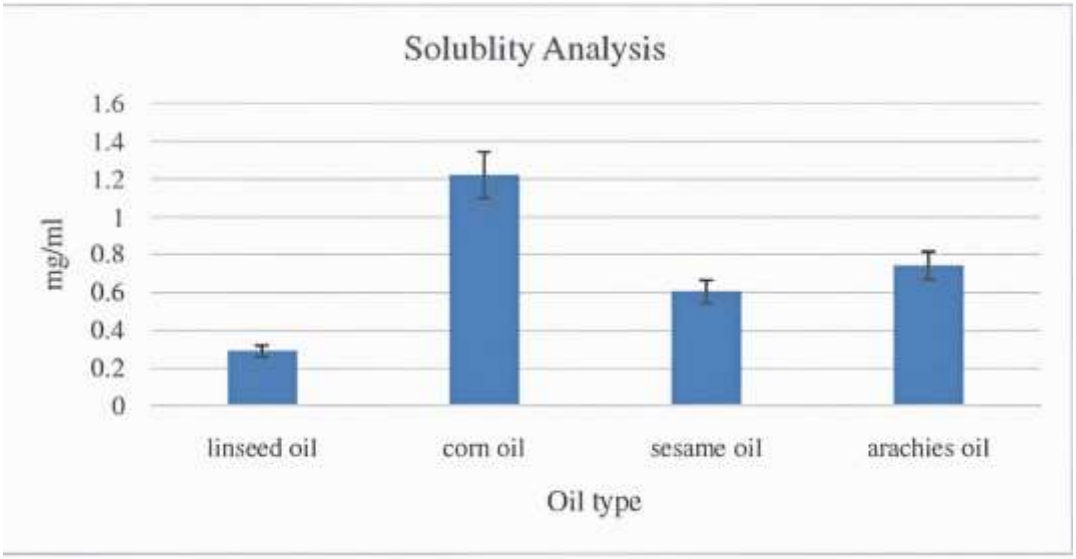


Figure No.2. Solubility of Centella asiatica extract in different oils.

Evaluation of herbal wound healing cream:

It was discovered that every developed formulation fell inside the bounds. It was discovered that the P8 formulation had the highest medication concentration. P8 formulation satisfies pharmaceutical parameter criteria. At 60°C, Glyceryl monosterate and Cetyl alcohol were melted in liquid paraffin to create the oil phase. Adding propyl paraben occurred during the same stage. Methylparaben was added to the phase in which aqueous Centella Asiatica Extract had been dissolved in maize oil. For the aqueous phase to develop, it was heated to 600C. Up until an emulsion was produced, the oil phase was introduced to the aqueous phase while being constantly stirred.

Table No.3. Design and evaluation of herbal cream**.**

Formulation	pH	Viscosity(cp)	Hardness(g.cm/sec)	Particle size	Drug content%
T1	6.67	3050	44.31	732.9	96.1
T2	6.65	1110	44.31	475	64.1
T3	6.45	1420	6.41	466.5	72.9
T4	6.97	1300	6.38	481	63.4
T5	6.00	2510	10.77	518.5	82.8
T6	6.25	1970	16.68	618.9	78.9
T7	7	2150	9.21	492.3	86.2

The formulation T6's allowed pH for topical application. Topical systems should have a pH that is compatible with the skin because they are administered directly to the skin.

TLC identification:

Following treatment with Anisaldehyde sulfuric reagent, the chromatographic profile of the cosmetic cream showed similar spots at RF and color with the profile of Centella asiatica extract solution. Lower on the chromatogram was a dark green spot (RF: 0.48) that corresponded to asiaticosides (Figure 2). Asiaticosides were utilized as a marker in the TLC analysis of the Centella asiatica raw material extract because of its lower cost and greater commercial availability. The primary active component of the recovered raw material, asiaticosides, was consequently discovered.

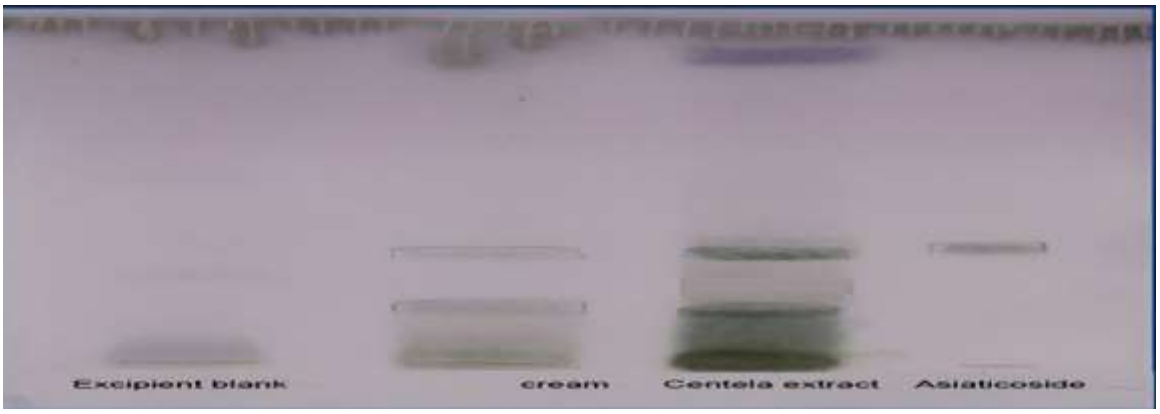


Figure No.3: TLC analysis. Excipients blank; Cream; Centella extract; Asiaticosides**.**

Summary:
The composition and assessment of an herbal cream intended to promote wound healing are the main topics of the study article. The study includes the selection of particular herbal extracts, such as Centella asiatica, that are well-known for their ability to heal wounds. A cream base is then combined with these extracts. Optimizing the concentration of herbal extracts and making sure the cream has the right physical attributes like consistency, Spreadability, and stability are two aspects
The of formulation process.

Several experiments are conducted to determine the herbal cream's safety and efficacy. Important assessment factors are the rate at which the wound contracts, tissue regeneration observed through histological investigations, and the absence of pollutants in the cream by microbiological investigations. In comparison to a typical control, the results show that the herbal cream dramatically accelerates wound .healing

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Conclusion:

Out of all the formulations, the T6 formulation or cream was determined to be the most optimal and fulfilling. When applied, it felt smooth and cool and had a light brown hue. The formulation's pH of 7.00 was determined to be suitable for the skin. Additionally, the creams demonstrated good hardness (9.21 g.cm/sec) when tested with a texture analyzer, which is a device made up of two glass cone probes. Measure the Hardness after adding 20 grams of cream to one probe, up to the top plane surface. With the help of this device, adhesive force and hardness may be compared and measured. The type of smear that appeared on the skin after the cream was applied was discovered to be non-greasy and easily removed with a tap water wash. The made cream was found to have a viscosity of 2150 cps at 25 rpm, indicating that it could be readily with minimal shea

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