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Formulation and Evaluation of Herbal Wound Healing Cream

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Abstract: *The purpose of the present research work is to formulated and evaluated herbal wound cream. The objectives of this research work are to formulate the cream which does not cause any adverse or side effects, with antimicrobial properties. The present study is to prepare and evaluated the herbal wound healing cream comprising extracts of natural products with Cucumber as the main active ingredient. The method utilized to prepare herbal cream is maceration method. The evaluations of all parameters like pH, stability study, spreadability studies and antimicrobial study were examined herbal formulations are effective in promoting the wound-healing process. They can stimulate a variety of physiological functions that accelerates the process of healing.*

Keywords: Wound healing, Anti-inflammatory, Cinnamon, Cucumber, Emulsion.

I. INTRODUCTION

A wound is any disruption of or damage to living tissue, such as skin, mucous membranes, or organs. Wounds can either be the sudden result of direct trauma (mechanical, thermal, chemical), or can develop slowly over time due to underlying disease processes such as diabetes mellitus, venous/arterial insufficiency, or immunological diseases . Wounds can vary greatly in their appearance depending on wound location, injury mechanism, depth of injury, timing of onset (acute vs chronic), and wound sterility, among other factors. Treatment strategies for wounds will vary based on the classification of the wound, therefore it is essential that wounds be thoroughly evaluated by a healthcare professional for proper management. In normal physiology, all wounds will undergo a series of steps collectively known as the wound healing process, which include hemostasis, inflammation, proliferation, and tissue remodeling. Age, tissue oxygenation, stress, underlying medical conditions, and certain medications are just a few of the many factors known to affect the rate of wound healing.[1] Wound healing is an important but complicated process in human or animal, containing a multifaceted process governed by sequential yet overlapping phases, including hemostasis/inflammation phase, proliferation phase, and remodeling phase.[1] After an injury to skin, the exposed sub-endothelium collagen and tissue factor will activate platelet aggregation which results in degranulation and releasing chemotactic factors (chemokines) and growth factors (GFs) to form the clot, and all above-mentioned procedures will achieve successful hemostasis human or animal, containing a multifaceted process governed by sequential yet overlapping phases, including hemostasis/inflammation phase, proliferation phase, and remodeling phase.[1] The wound healing assay is a common in-vitro method used to study how cell migrate collectively.[2] Natural and synthetic biomaterials are widely used in wound care due to their biocompatibility and ability to promote cell growth .wound healing is a vital process involving tissue repair with the aim of the closing the wound quickly while reducing pain and scarring. A wound is essentially break in the skin protective barrier caused by injury, temperature changes, pressure, diseases.[3]

CLASSIFICATION OF WOUNDS

Wounds can be broadly classified as either acute or chronic based on time from initial injury and progression through normal stages of wound healing. Both wound types can further be categorized by cause of injury, wound severity/depth, and sterility of the wound bed.[4]

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A. Acute wound

An acute wound is any wound which results from direct trauma and progresses through the four stages of wound healing along an expected timeline. The first stage, hemostasis lasts from minutes to hours after initial injury. This stage is followed by the inflammatory phase which typically lasts 1 to 3 days. Proliferation is the third stage of wound healing and lasts from a few days up to a month. The fourth and final phase of wound healing, remodeling/scar formation, typically lasts 12 months but can continue as long as 2 years after the initial injury. [5]

Acute wounds can further be classified as either open or closed.

• An open wound is any injury whereby the integrity of the skin has been disrupted and the underlying tissue is exposed.

• A closed wound, on the other hand, is any injury in which underlying tissue has been damaged but the overlying skin is still intact.[6]

B. Chronic Wound

Any wound which is arrested or delayed during any of the normal stages of wound healing is considered to be a chronic wound . Most commonly, these are wounds which develop due to an underlying disease process such as diabetes mellitus or arterial/venous insufficiency. However, it's important to note that any acute wound has the potential to become a chronic wound if any of the normal stages of wound healing are interrupted.

Common causes of chronic wounds

• Diabetes mellitus
• arterial insufficiency
• Immunologic disease
• Pressure ulcer

Wound Sterility

Wound sterility, or degree of contamination of a wound, is a critical consideration when evaluating a wound. According to this classification system, 4 different classes of wound exist, each with their own postoperative risk of surgical site infection.

• Class 1 - clean wound: a wound that is not infected and without signs of inflammation. This type of wound is typically closed. By definition, this type of wound excludes any wounds of the , genital, respiratory, alimentary, urinary tract

• Class 2 - clean-contaminated wound: a wound with a low level of contamination. May involve entry into the respiratory, genital, alimentary, or urinary tract.

• Class 3 – contaminated wound: an open, accidental wound resulting from trauma outside of a sterile setting is automatically considered a contaminated wound. Additionally, any surgical wound where there is a major break in sterile technique or obvious contamination from the gastrointestinal tract is considered a contaminated wound.

• Class 4 – dirty/infected: a wound with evidence of an existing clinical infection. Class 4 wounds are usually found in old traumatic wounds which were not adequately treated and will show evidence of devitalized tissue or gross purulence.[6]

PHYSICAL EXAMINATION OF WOUND HEALING

Wound presentation will vary greatly based on a number of factors, each of which is important to consider in order to establish a proper diagnosis and treatment plan. In addition to collecting a thorough history, the following factors should be considered when evaluating any wound:

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• Size of wound: Should be accurately measured at time of initial presentation and regularly remeasured until wound resolution.

1. Wound location: Very useful consideration in many chronic wounds, such as diabetic food ulcer, venous ulcer. Acute wounds will be located in areas consistent with the mechanism of injury (e.g. diagonal chest wall bruising from seatbelt following car accident.

2. Wound bed: A healthy wound bed will appear pink due to health granulation tissue. Presence of a dark red wound bed which bleeds easily on contact or excess granulation tissue (i.e. hyper granulation tissue) may indicate the presence of an infection or non-healing wound.

3. Wound depth: The depth of a wound is often not apparent on visual inspection alone. Proper evaluation of wound depth includes use of a probe to measure wound depth and evaluate for undermining of wound edges or sinus formulation.

4. Necrotic tissue, slough, Wounds may be covered with a layer of dead tissue which may appear cream/yellow in color (slough) or as a black, hardened tissue. Removing this tissue is critical for properly evaluating both the depth of a wound and quality of the wound bed, and promotes wound healing.

5. Wound edges: May provide clues to cause of specific wounds, such as gently sloping edges of venous ulcers or rolled edges of certain tumors.

6. Surrounding skin: Appearance of the surrounding skin can provide clues to underlying disease processes, such as redness /erythema to cellulitis maceration due to uncontrolled wound exudate or eczematous changes due to a chronic irritation (e.g. allergic reaction wound dressing).

Infection; Classic signs of infection are redness, warmth, swelling, odor, and pain out of proportion to wound appearance.[7]

7. Pain; Pain can be nociceptive or inflammatory, each of which can provide clues to the cause of a wound. Proper pain controls an important consideration in wound management, particularly in burn care where analgesia is often necessary prior to dressing changes.

DIAGNOSIS OF WOUND

• Wound culture; If there is concern for infection, a wound can be more carefully evaluated for presence of bacteria via surface swabs, deep tissue biopsy , or needle biopsy. Surface swabs are most commonly used due to low cost, ease of use, and minimal pain to patient.

• Imagine X ray is useful to assess for an underlying fracture which may not be apparent on physical examination alone. Ultrasound , Computed sonography (CT), and Magnetic Resonance Imaging (MRI) can all be used to assess for identifying fluid collections, necrotic tissue, or inflammation. Ultrasound is portable, low cost, quickly implemented, and does not expose patients to radiation , but is limited in diagnostic capabilities.

• Laboratory studies: Serum prealbumin levels may be useful in evaluating nutrition status in patients with chronic wounds or at risk for developing chronic wounds.

• Ankle-brachial index/toe-brachial index (ABI/TBI): These tests can be used to assess blood supply to the lower extremities and their results may affect management of lower extremity wounds such as venous/arterial ulcers, diabetic foot ulcers, or pressure ulcers.

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PATHOGENESIS OF WOUND HEALING

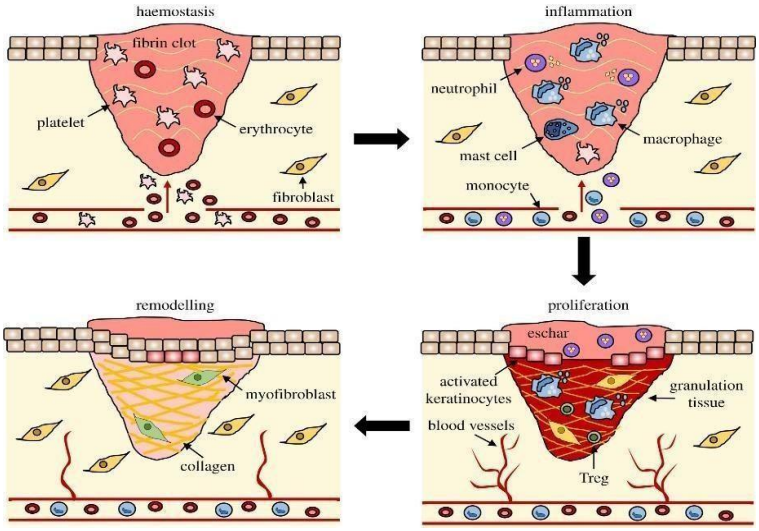

Fig.1. Pathogenesis of skin

Inflammation

This phase includes hemostasis and inflammation. An injury to the skin immediately initiates clotting cascades which provide a temporary fibrin blood clot plug to the injury site. Meanwhile, 5- to 10-minute vasoconstriction is triggered in the wounded area. These temporary reactions prevent further bleeding and protect the wound. Moreover, this fibrin plugs forms a temporary matrix which serves as a scaffold structure for further healing processes like the migration of leukocytes, keratinocytes, fibroblasts, endothelial cells and

serves as a growth factor resource. Vasodilatation occurs after this brief vasoconstriction response which will cause local hyperemia and edema.[8]

Proliferation

The following proliferative phase is characterized by granulation tissue formation and vascular network restoration. This phase starts approximately 3 to 10 days after injury and takes days or weeks to complete. Various cytokines and growth factors have a role in this phase such as transforming growth factor-beta family (TGF-beta, including TGF-beta1, TGF-beta2, and TGF-beta3), interleukin (IL) family and angiogenesis factors. The predominate proliferating cells are fibroblasts and endothelial cells in this phase. During the cell proliferation, a requirement for an adequate blood supply occurs. Therefore, an angiogenic response is initiated simultaneously. This response is mainly stimulated by local hypoxia, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor-basic (FGF) and the serine protease thrombin. New vessels are built up by 2 mechanisms which are angiogenesis and vascular genesis. Angiogenesis is a “sprouting” process in which neo-vessels grow into the avascular site from resident endothelial cells of the adjacent mature vascular network. However, vascular genesis is a de novo process in which progenitor stem cells differentiate and form new vessels without “sprouting” from any mature vascular network.[8]

Remodeling

Remodeling is the last phase of the wound healing, begins from day 21 and continues up to 1 year. In this phase, there is a precise balance between synthesis and degradation of the new tissue that needs to be strictly preserved. Any disruption ends up with a chronic wound formation. During the remodeling phase, the granulation tissue formation

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ends, and the maturation of the wound begins. ECM components exposed to some certain modifications to form a stronger and organized ECM. Collagen type III is replaced by stronger collagen type 1. The tensile strength of the wound gradually increases. The collagen synthesis continues for at least 4 to 5 weeks. However, the collagen in the wounded area will never be as organized as collagen found in the healthy skin. It is important to note that during collagen synthesis the hydroxylases require oxygen and vitamin C.

PHYSIOLOGY OF SKIN

1. Epidermis

The epidermis is the most superficial layer of the skin and is composed of stratified keratinized squamous epithelium, which varies in thickness in different parts of the body. It is thickest on the palms of the hands and soles of the feet. There are no blood vessels or nerve endings in the epidermis, but its deeper layers are bathed in interstitial fluid from the dermis, which provides oxygen and nutrients, and drains away as lymph.[9]

2. Dermis

The dermis is tough and elastic. It is formed from connective tissue and the matrix contains collagen fibers interlaced with elastic fibers. Rupture of elastic fibers occurs when the skin is overstretched, resulting in permanent striae, or stretch marks, that may be found in pregnancy and obesity. Collagen fibers bind water and give the skin its tensile strength, but as this ability declines with age, wrinkles develop. Fibroblasts, macrophages and mast cells are the main cells found in the dermis. Underlying its deepest layer there is areolar tissue and varying amounts of adipose (fat) tissue.[9]

3. Subcutaneous gland

These consist of secretory epithelial cells derived from the same tissue as the hair follicles. They secrete an oily substance, sebum, into the hair follicles and are present in the skin of all parts of the body except the palms of the hands and the soles of the feet. They are most numerous in the skin of the scalp, face, axillae and groins. In regions of transition from one type of superficial epithelium to another, such as lips, eyelids, nipple, labia minora and glans penis, there are sebaceous glands that are independent of hair follicles, secreting sebum directly onto the surface.[9]

OBJECTIVES

Primary objective

- To formulate and evaluate a topical wound healing cream containing Cucumis sativa that effectively promotes skin repair and accelerates the wound healing process.

Secondary objective

- To study the physiochemical properties.
- To assess the stability.
- To evaluate the wound healing activity.
- To ensure safety and non-irritancy.
- To optimize the formulation for better absorption and patient compliance.

NEED OF WOUND HEALING CREAM FORMULATION FROM CUCUMIS SATIVA

- Stability of active constituents

Fresh cucumber contains bioactive compounds such as flavonoids.

- Controlled and sustained drug release

A cream base ensures gradual release of active constituents and better therapeutic effect.

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• Enhanced therapeutic activity

Cucumis sativa provides anti-inflammatory action, antioxidant effect, cooling and soothing effect and mild antimicrobial activity.

• Safety and reduced side effect

Herbal creams are less irritating than synthetic drugs and suitable for sensitive skin.

• Cosmetic and patient acceptability Cream formulation improve texture and appearance.

• Ease of application

Compared to crude extracts cream are easy to apply and spread, non-greasy.

LITERATURE AND SURVEY

SR.NO	Author	Title	Summary
1.	Timothy F. Herman; Bruno Bordoni (National library of medicine ,2023)	Wound classification	A Wound forms when biological tissue like skin, mucous membranes and organs are damaged. Different injuries can cause wound; properly cleaning and dressing the wounds is essential to prevent infection and additional harm.
2.	Pena, O.A., Martin. P (Natural reviews Molecular cell Biology,2024)	Cellular and molecular mechanism of skin wound healing	We highlight the contribution of different cell types to skin repair, with emphasis on how both innate and adaptive immune cells in the wound inflammatory response influence classically studied wound cell lineages, including keratinocytes , fibroblasts and endothelial cells, but also some of less studied cell lineages such as adipocytes, melanocytes and cutaneous nerves.
3.	Turaga Amani, Mouttoukichenin Surenthar Rajeshkumar Shanmugam (National library of Medicine)	Anti-inflammatory and Antioxidant activity of Cucumis sativa and Citrus macropterous herbal formulation; An In-vitro study.	Current research is focused on examining the antioxidant and anti-inflammatory characteristics of a combination of Cucumis sativa and citrus macropterous extract in an in-vivo context.
4.	Chhabra S. Chhabra N. Kaur A. Gupta N (Journal of Maxillofacial and Oral Surgery, December 2017)	Wound Healing Concepts in Clinical Practice of OMFS	This review article describes the classification of wound and aims to highlight the fundamentals of wound repair enumerating the dressing used commonly and also, the newer concepts of wound healing.
5.	Vaughn AR. Branum A. Sivamani RK. (Pubmed,2016)	Effects of Turmeric (Curcuma longa) on skin health; A systematic review of the clinical evidence.	Turmeric (Curcuma longa), A commonly used spice throughout the world, has been shown to exhibit anti-inflammatory, antimicrobial , antioxidant properties.

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DRUG PROFILE

Synonyms: Cucumber
Biological source: Fruit is derived from plant Cucumis sativa.
Family: Cucurbitaceae
Chemical Constituents:
Phytoconstituents- Flavonoids, Tannis Vitamins- Vitamin C, Vitamin K Minerals- Potassium, Magnesium, Silica
Useful part: Fruits
Taste: Mild, slightly sweet
Color: Green
Odor: Non-aromatic
Therapeutic use: Cooling effect, Anti-inflammatory, Anti-acne

Fig.2. Cucumber

MATERIAL AND METHODS

SR.NO	INGREDIENTS	ROLE
1.	Cucumber extract	Anti-inflammatory
2.	Turmeric powder	Antioxidant
3.	Stearic acid	Emulsifying agent
4.	Beeswax	Thickening agent
5.	Cetyl alcohol	Stabilizer
6.	Glycerine	Humectant
7.	Methyl paraben	Preservative
8.	Triethanolamine	PH adjuster
9.	Distilled water	Vehicle

Table No.1: Ingredients of wound healing cream

METHODOLOGY

1. Collection of Raw Material:

The raw materials for the herbal wound-healing cream were obtained from authenticated sources, and the cucumber fruit were procured from a reputable herbal supplier and verified by a qualified botanist.

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2. Maceration Method

1. Extraction of Cucumis sativus
2. 50 gm of fresh cucumber is firstly wash.
3. Slice the cucumber into thin parts.
4. Put cucumber slices into the sterile glass jar.
5. Add 50gm of vegetable glycerine in the glass jar.
6. Mix it for few seconds
7. Notice that the cucumber is fully covered with glycerine.
8. Seal the jar and keep it for 10 Days, away from sunlight.
9. After 10 days strain the cucumber extract by using Cheese cloth.[10]

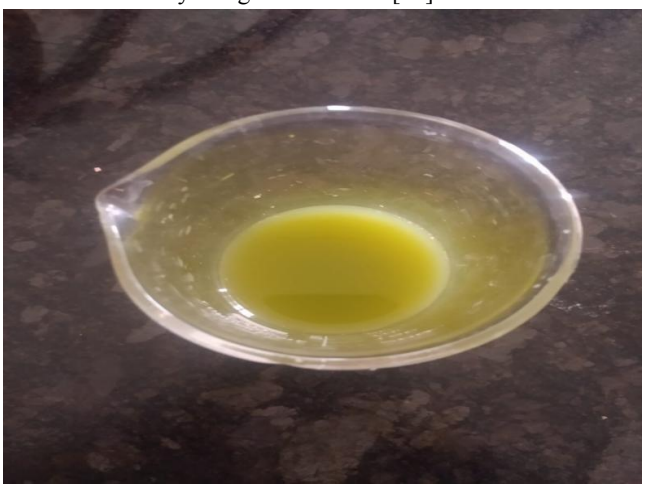

Fig .3. Cucumber extract

3. Preliminary test of cucumber extract

1. Test for triterpenoids: 2ml of Trichloroacetic acid was added to 1ml of extract. The presence of terpenoids was confirmed by the formation of red precipitate.
2. Test for Tannis: 0.5gm of plant extract and 2ml of water then heat on water bath, filter all 1 ml10% fecl3, formation of blue-black solution.
3. Test for Flavonoids:5ml Distilled water + 0.2gm extract mixed add 1ml of 1% Alcl3, formation of light-yellow ppt.
4. Test for Amino Acid: 0.2gm extract+5ml distilled water mixed left for 3hrs than filter, 2ml filtrate+0.1 ml million reagents. Formation of yellow.
5. Alkaloids: 3ml conc. extract+1ml HCL heat for 20min cooled and filter. Formation of brown reddish ppt.
6. Phenols: extract+4 drops alc. Fecl3 sol. Formation of bluish black color.

Preliminary test of Cucumber extract

NO.	TEST	OBSERVATION	RESULT
1.	Flavonoids Test	Light yellow	+
2.	Tannin Test	Blue-black solution	+
3.	Amino Test	Yellow	+
4.	Terpenoids Test	Red precipitate	+
5.	Saponin test	Milky white ppt	+

Table No: 2 Preliminary test of Cucumber extract

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Fig .4. Preliminary test of Cucumber extract

FORMULATION TABLE

SR.NO	INGREDIENTS	F1 (30 gm)	F2 (30 gm)
1.	Cucumber extract	1.50 ml	2.00 ml
2.	Turmeric powder	0.45 gm	0.85 gm
3.	Stearic acid	3.75 gm	3.00 gm
4.	Beeswax	1.05 gm	2.25 gm
5.	Cetyl alcohol	1.20 gm	1.50 gm
6.	Methyl Paraben	0.45 gm	0.45 gm
7.	Glycerine	1.80 ml	1.50 ml
8.	Triethanolamine	0.45 ml	0.38 ml
9.	Distilled Water	q. s	q. s

Table No. 3 Formulation Table of Cream

PROCEDURE:

Step 1: Preparation of Oil Phase

Take stearic acid, cetyl alcohol, and beeswax in a beaker and heat to 70–75°C until completely melted. [11,12]

Step 2: Preparation of Aqueous Phase

Dissolve glycerine and methyl paraben in distilled water and heat to the same temperature [13]

Step 3: Emulsification

Add the oil phase slowly into the aqueous phase with continuous stirring to form an emulsion. [12,13]

Step 4: Neutralization & Cream Formation

Add triethanolamine (TEA) gradually with stirring.[15]

Step 5: Cooling Phase

Allow the cream to cool to below 40°C, then add: Cucumber extract and Turmeric extract. [14,15]

Step 6: Final Adjustment

Adjust pH to 5.5–6.5, mix thoroughly, and ensure uniform consistency.[16]

Step 7: Packaging

Transfer into clean, sterile containers and store properly.[16]

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Fig. 5. Wound healing cream

EVALUATION PARAMETERS

1. Physical evaluation

The wound healing cream was tested for odor, appearance and homogeneity. Color - The color of the cream was observed by visual examination i.e. yellow color Odor - The odor of cream was found to be characteristics.

Appearance - The appearance of cream was examined visually.

Consistency - The formulation was examined by rubbing cream on hand manually.

Fig. 6. Wound healing cream

2. PH of cream:

The pH of the Wound healing cream was determined using a Digital pH meter.

1gm of sample dissolve into 10ml of distilled water and then electrical rod dipped into this solution further check the pH of cream.[17]

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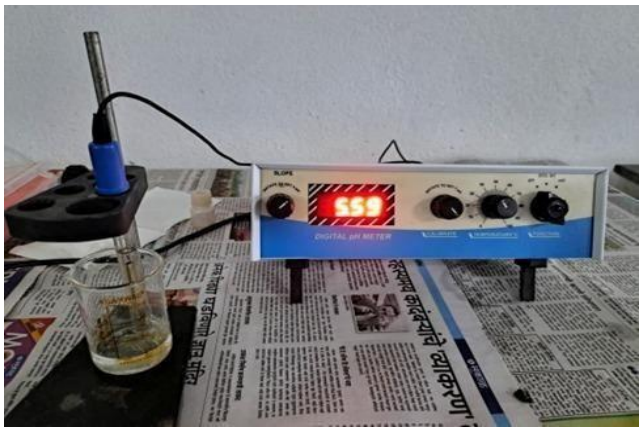

Fig. 7. PH of cream

3. Washability test

A. Application:

Apply a measured amount of wound healing cream(e.g.,1gm) evenly on a glass slide or small skin area. Allow it to sit for 30 minutes to measure absorption time.

B. Washing:

Wash the area using.

Distilled water +Mild soap +water.

Use a wet cotton ball or sponge and gently rub the area in a consistent circular motion.[18]

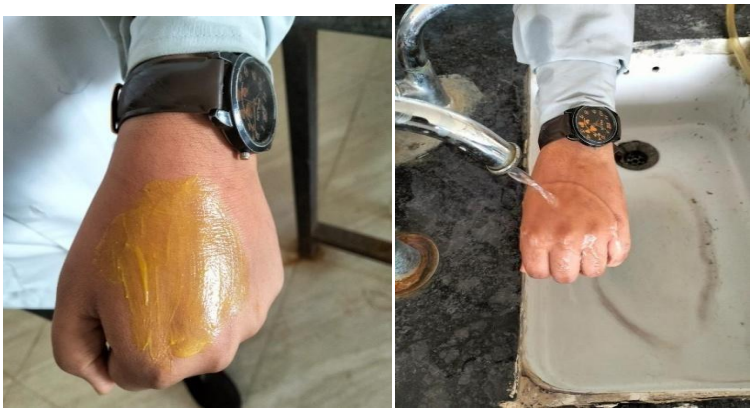

Fig .8. Washability of cream

4. Spreadability Test:

Take 1gm of Wound healing Cream between glass slide, forming a thin layer. Take 20gm of weight on the Top Slide for 5min to allow uniform spreading. Then remove weight Record the time (seconds) it takes foe upper slide to move a fix distance.[19] Spreadability can be expressed as,

S=m X L /T(sec)

Where,

m= weight applies to upper slide

L = length moved on the glass slide T= Time take

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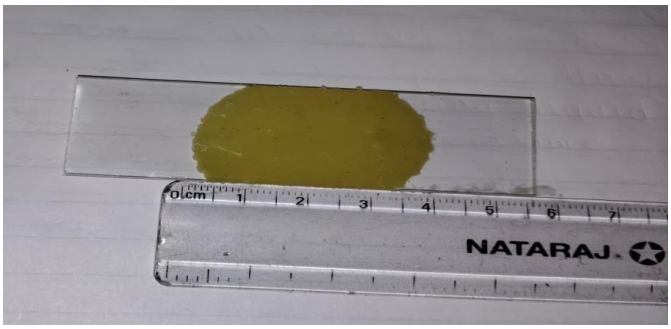

Fig. 9. Spreadability of cream

5. Irritability test:

Take the small amount of sample and applied on the hand and kept the cream as it is for 30 min to observe the Redness, Swelling and Irritation on hand.[20]

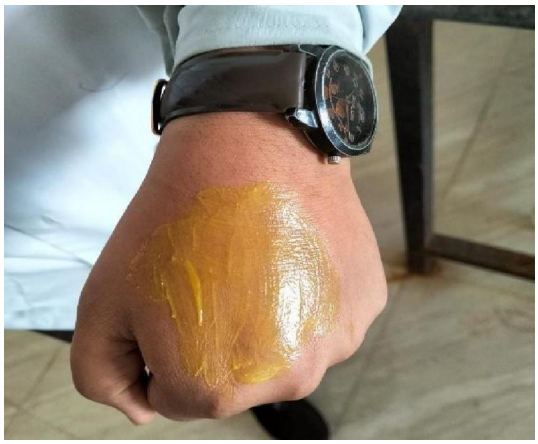

Fig. 10. Irritation testing of cream

6. Homogeneity:

The formulation was tried for the uniformity by visual appearance and by touch.[21]

7. Stability Study:

Store the wound healing cream at different conditions and check it periodically for changes in appearance, pH, viscosity, and stability. Record any changes to determine whether the cream remains stable over time.[21]

8. Viscosity test:

The viscosity of wound healing cream is determined by using Brookfield viscometer.[22]

Procedure:

Mix the cream sample gently and maintain at 25 °C.
Fill the sample in a clean beaker without air bubbles.
Select suitable Brookfield spindle (commonly RV spindle for creams). Set required speed (e.g., 10 rpm).
Start the viscometer and allow reading to stabilize.

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Record:
Viscosity (cP/mPa·s) Spindle number Speed (rpm) Temperature
Torque %

Fig. 11. Viscosity of cream

9. In vitro test

An in vitro study of a wound healing cream evaluates the cream's biological activity under laboratory conditions using cultured cells, artificial membranes, or biochemical assays
before animal or human testing.[23] The report of in vitro testing is included in result.

• Evaluation studies of formulation batches

SR.NO	EVALUATION PARAMETER	F1	F2
1.	Appearance	White	Yellow
2.	pH	7.06	5.59
3.	Washability	Washable	Washable
4.	Spreadability	0.31 cm	0.2 cm
5.	Irritability	No Irritable	No Irritable
6.	Homogeneity	Homogenous	Homogenous
7.	Stability	Stable	Stable
8.	Viscosity	30000 cp	40000 cp

Tabel No.4 Evaluation studies of formulation batches

The comparative evaluation of the two wound-healing cream formulations clearly demonstrates that the F2 formulation exhibited superior performance over F1 across all tested parameters. The graph shows consistently higher percentages for F2 in moisturizing effect, wound-healing rate, anti-inflammatory activity, skin softness, spreadability, and overall effectiveness.

The improved performance of F2 can be attributed to its optimized composition, particularly the increased amount of moisturizing agents such as glycerine and the softer cream base. These modifications enhanced the hydration capacity of the formulation, which is an important factor in promoting faster wound repair and maintaining healthy skin tissue around the wound site.

F2 also showed better anti-inflammatory activity, indicating that the formulation may provide improved soothing action and reduction of irritation at the wound area. Enhanced spreadability and skin softness further suggest that F2 offers better patient comfort and easier topical application compared to F1.

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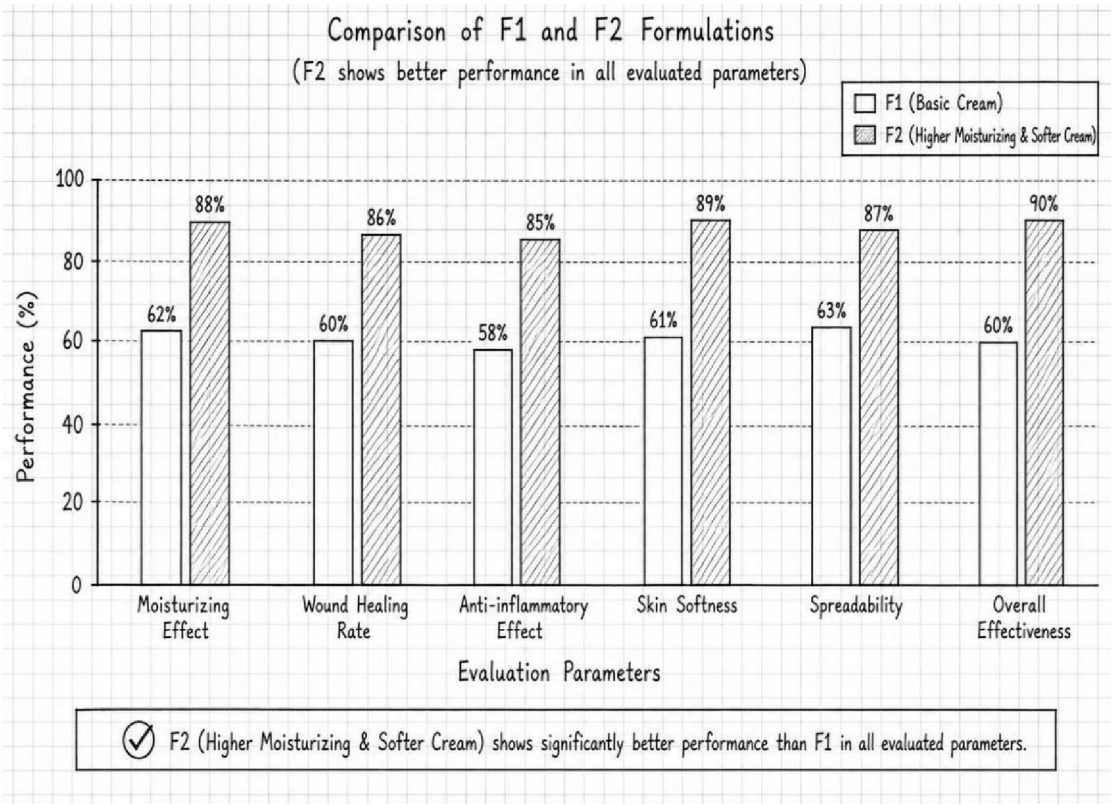

The higher wound-healing percentage observed in F2 indicates that the formulation may support faster tissue regeneration and improved recovery. Proper moisture retention created by the cream likely contributed to maintaining an ideal environment for wound healing.

Overall, the results confirm that the F2 formulation is more effective, stable, and cosmetically acceptable than the F1 formulation. Therefore, F2 can be considered a promising wound-healing cream with enhanced therapeutic and skin-conditioning properties suitable for topical application.

RESULT AND DISCUSSION

The present research is the formulation and evaluation of Herbal wound healing cream. The evaluation parameters are coming under results, like the physical evaluation of polyherbal cream, pH of the cream. Spreadability, Washability, non-irritancy test, viscosity and phase separation of the wound healing cream.

Physical properties: The physical properties of formulated cream are judged by color, odor and texture. (Yellow color is observed.)

Washability: The cream applied on skin is easily removed by washing with tap water.

pH of the cream: The pH of the cream is found to be in range of 5.6 to 6.8 which is good for skin pH. The herbal formulation is shown pH nearer to skin required i.e. pH 6.8.

Viscosity: Viscosity of formulated cream is determined by brook field viscometer at 20 rpm The viscosity of cream is in the range of 35000 to 50000 cp which indicates that the cream is easily spreadable by small amount of shear. The formulated cream shows the viscosity.

Spreadability test: The spread ability test showed that the formulated cream has good spreadable property.

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Irritancy test: The formulated cream shows no redness, edema, irritation and inflammation during during studies. The formulated cream is safe to use.

Homogeneity: The formulation is tested for the homogeneity by visual appearance and by touch.

IN-VITRO STUDY REPORT

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Ref: BiRD/1228/12/2025 **Date: 02/05/2026**

A) Name of Client	**Ms. Tanuja Jotiba Katkar.**
B) Name of College or Organization	**Nootan College of Pharmacy Kavathe Mhankal.**
C) Sample Description	**Sample – F2**
D) Sample Form	**Semi solid**
E) Activity:	**In vitro Scratch assay**
F) Cell line:	**L929 fibroblast cell line**
G) Media	**DPBS, FBS, Antibiotic – Antimycotic solution**

Scratch assay

The wound healing capabilities of Sample was assayed by performing In vitro cell migration studies on L929 cells by a previously described method. Briefly, 2 x 10^5 cells/mL were seeded in 6- well plates and were cultured overnight. Cells were then washed with Dulbecco's Phosphate Buffered Saline (DPBS) and a scratch was made with a sterile 200µL tip. The detached cells and other cellular debris were removed by washing the cells with DPBS. The cells were treated with 100 µL of Sample and 5 µg/mL of positive control, Cipladine and incubated for 24 h. Cipladine is a standard drug that is used in wound healing. Untreated cells were negative control. The cell migration and morphological changes of cells were observed in the images taken by inverted microscope, equipped with digital camera. The experiments were performed in triplicate (n ¼ 3). The width of the scratch and wound closure at different time intervals (48hrs) was analyzed by SAGLO software.

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II. CONCLUSION

From the above results it is concluded that the formulated cream showed good consistency and spread ability, homogeneity, pH, non-greasy and there is no phase separation during study period of research. From the above study it can be concluded that the polyherbal cold cream is safe to use as it is developed from herbal extract and may be applied topically against scalp psoriasis. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. So, the values of herbs in the cosmeceutical have been extensively improved in personal care system and there is a great demand for the herbal cosmetics nowadays. An herbal cream which is non-toxic, safe, effective and improves patient compliance by the utilization of herbal extracts would be highly acceptable than synthetic ones. The Cucumis sativus, Curcuma antibacterial, anti-inflammatory, antiseptic and wound healing property which helps to heal wound. Formulation of cream is done by maceration method and further evaluated by various evaluation parameters such as physical properties, pH, Spreadability, Washability, non-irritancy test, viscosity and phase separation of cream and gives good results.

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