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Evaluation of Herbal Formulation for Burn wounds

Momin Asad Mohammad Murtuza, Prof. Komal A. Dongare, Dr. Surwase K. P
Aditya Institute of Pharmaceutical, Beed

Abstract: *Burn wounds are one of the most common and severe forms of skin injury that may lead to inflammation, microbial infection, tissue damage, fluid loss, and delayed healing. Conventional burn wound treatments such as antibiotics and synthetic topical preparations are associated with several limitations including skin irritation, allergic reactions, high treatment cost, and development of antibiotic resistance. Herbal medicines have gained increasing attention due to their therapeutic effectiveness, safety, affordability, and minimal side effects.*

The present study was undertaken to formulate and evaluate a herbal topical preparation for burn wound healing activity using medicinal plant extracts such as Aloe vera, Neem, and Turmeric. These medicinal plants are well known for their antimicrobial, anti-inflammatory, antioxidant, and tissue regenerative properties. Herbal extracts were prepared by Soxhlet extraction method using suitable solvents and incorporated into an ointment base to develop a stable herbal formulation. The prepared formulation was evaluated for various physicochemical parameters including color, odor, consistency, pH, spreadability, viscosity, extrudability, and stability. Antimicrobial activity was evaluated using agar well diffusion method against selected pathogenic microorganisms. Burn wound healing activity was assessed using experimental animal models by studying wound contraction, epithelialization period, and histopathological changes.

The results demonstrated that the herbal formulation possessed satisfactory physicochemical characteristics with good stability and spreadability suitable for topical application. Significant antimicrobial activity and enhanced burn wound healing activity were observed compared to control group. Faster wound contraction, reduced inflammation, improved collagen synthesis, and accelerated tissue regeneration were observed during the study..

Keywords: Burn Wound Healing, Herbal Formulation, Aloe vera, Neem, Turmeric, Antimicrobial Activity, Wound Contraction, Herbal Ointment, Tissue Regeneration, Phytoconstituents.

I. INTRODUCTION

Burn wound is one of the most traumatic and serious forms of injury affecting millions of individuals globally every year. A burn injury causes destruction of skin tissue due to exposure to thermal, chemical, electrical, radiation, or frictional agents. Skin acts as the first protective barrier of the human body and protects internal organs from microbial invasion, dehydration, and environmental damage. Damage to the skin therefore leads to severe physiological and pathological complications.

Burn injuries are associated with:

- Pain
- Inflammation
- Tissue necrosis
- Fluid loss
- Infection
- Delayed wound healing
- Scar formation

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Burn wounds are considered a major public health problem, especially in developing countries where access to advanced medical treatment is limited. Proper wound management is essential to reduce complications and promote rapid healing.

The severity of burn injury depends upon:

- Temperature of the burning agent
- Duration of exposure
- Area of body affected
- Depth of injury
- Age and health condition of patient

Structure of Skin

The skin is the body's largest organ, serving as a vital barrier against pathogens, UV light, and chemical injury while regulating body temperature. It is composed of three primary layers :

1. Epidermis (Outermost Layer)

The epidermis is the tough, waterproof outer layer that determines skin tone and protects underlying tissue. It contains no blood vessels and is divided into several sub-layers:

Stratum Corneum: The dead outermost layer of flattened cells that sheds and acts as the primary barrier.

Stratum Lucidum: A clear, thin layer found only on the palms of the hands and soles of the feet.

Stratum Granulosum: Where cells begin to flatten and die as they move toward the surface.

Stratum Spinosum: Provides strength and flexibility.

Stratum Basale: The deepest layer where new skin cells (keratinocytes) are continuously generated.

Key cells: Includes melanocytes (produce melanin for skin color) and Langerhans cells (immune defense)

Functions:

Acts as a protective barrier: The epidermis keeps bacteria and germs from entering body and bloodstream and causing infections. It also protects against rain, sun and other elements.

Regeneration of skin: The epidermis continually makes new skin cells. These new cells replace the approximately 40,000 old skin cells that body sheds every day.

Protection: Langerhans cells in the epidermis are part of the body’s immune system. They help fight off germs and infections.

Provides skin color: The epidermis contains melanin, the pigment that gives skin its color. The amount of melanin determines the color of skin, hair and eyes. People who make more melanin have darker skin and may tan more quickly.

2. Dermis (Middle Layer)

Located directly beneath the epidermis, the dermis provides structure, elasticity, and support. It is made of dense, irregular connective tissue comprising collagen and elastin fibers. It houses critical structures:

Has collagen and elastin: Collagen is a protein that makes skin cells strong and resilient. Another protein found in the dermis, elastin, keeps skin flexible. It also helps stretched skin regain its shape.

Functions:

Blood and Lymph Vessels: Blood vessels in the dermis provide nutrients to the epidermis, keeping the skin layers healthy.

Nerve Endings: Responsible for transmitting sensations like touch, pain, pressure, and temperature to the brain.

Hair Follicles: The roots of hair follicles attach to the dermis.

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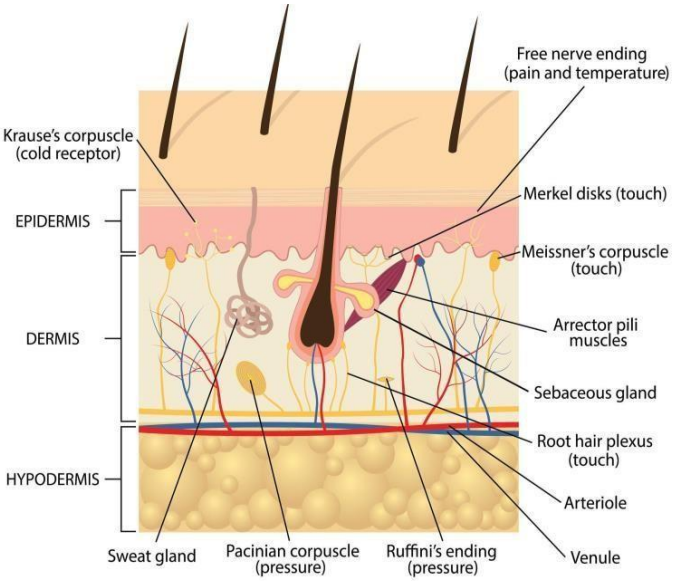
Sebaceous Glands: Oil glands in the dermis help keep the skin soft and smooth. Oil also prevents skin from absorbing too much water during swimming or get caught in a rainstorm.
Sweat Glands: Produce sweat to cool the body.

3. Hypodermis / Subcutaneous Tissue (Deepest Layer)
The hypodermis is the innermost layer acting as the anchor between the skin and underlying muscles and bones. It is composed primarily of loose connective tissue and fat (adipose tissue).
Cushions muscles and bones: Fat in the hypodermis protects muscles and bones from injuries when you fall or are in an accident.
Has connective tissue: This tissue connects layers of skin to muscles and bones.
Helps the nerves and blood vessels: Nerves and blood vessels in the dermis (middle layer) get larger in the hypodermis. These nerves and blood vessels branch out to connect the hypodermis to the rest of the body.
Regulates body temperature: Fat in the hypodermis keeps you from getting too cold or hot.



Burns
Damage to these layers during burn injury disrupts normal skin function and delays tissue repair.

Classification of Burns
Burns are classified according to the depth of tissue injury.

First Degree Burns
• Affect only epidermis
• Cause redness and pain
• Heal without scar formation

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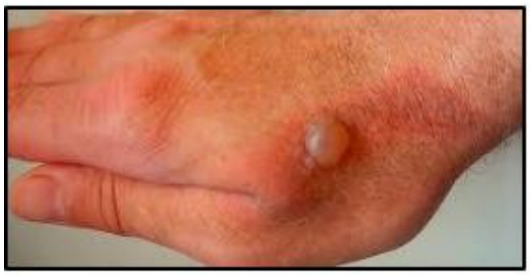
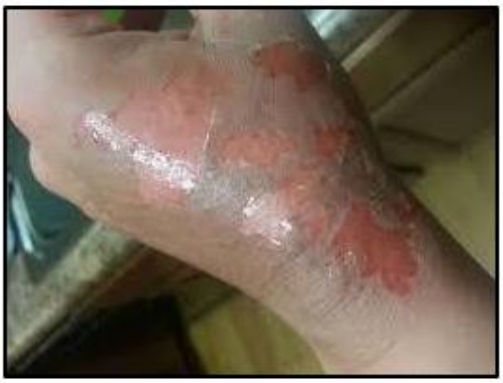


First Degree Burns

Second Degree Burns
- Affect epidermis and dermis
- Characterized by blister formation
- Painful and prone to infection



Second Degree Burns

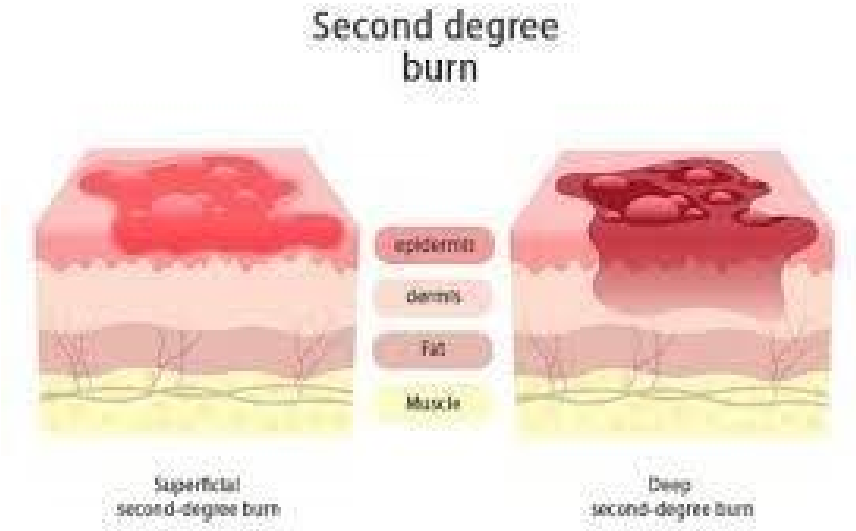


Fig. Difference Between Second degree superficial and deep burn

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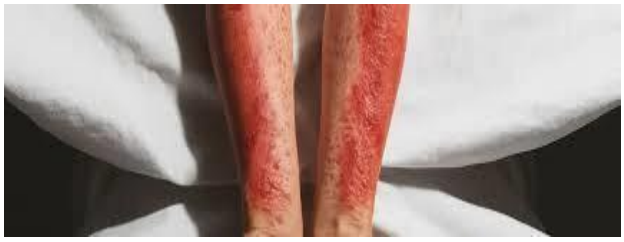
Third Degree Burns
• Extend into deeper tissues
• Cause destruction of nerves and blood vessels
• Require prolonged treatment and skin grafting



Third Degree Burns

Wound Healing Process

Wound healing is a complex biological process involving cellular and biochemical events.

1. Hemostasis Phase

Immediately after injury blood clot formation occurs to stop bleeding.

2. Inflammatory Phase

Inflammatory cells remove damaged tissue and microorganisms.

3. Proliferative Phase

Fibroblast proliferation and collagen synthesis occur resulting in granulation tissue formation.

4. Remodeling Phase

Maturation of collagen fibers and scar formation take place. Any interruption in these phases may delay healing

Conventional Treatment of Burn Wounds

The conventional treatment of burn wounds is a multi-step clinical process designed to stop the burning process, alleviate pain, prevent fluid shift/burn shock, minimize infection, and optimize functional and aesthetic recovery (Kim et al., 2022). Standard protocol divides care into immediate first aid, systemic stabilization, non-surgical wound management, and surgical interventions based on the depth and extent of the burn.

1. Immediate First Aid and Evaluation

The primary objective during initial contact is to stop the thermal insult and stabilize the patient.

Cooling: Immediate cooling of the burned area with water at an optimal temperature of 10–20°C is crucial to reduce tissue temperature and stop the progression of the thermal injury.

Ice must be strictly avoided as it induces vasoconstriction and exacerbates tissue ischemia.

Assessment of Severity: Burn severity is measured by depth (Superficial, Partial-Thickness, or Full-Thickness) and the Total Body Surface Area (TBSA) affected, frequently evaluated in adults using the Wallace "Rule of Nines".

2. Systemic Management: Fluid Resuscitation

For severe burns exceeding 15–20% TBSA, rapid systemic stabilization takes precedence over local wound care to prevent hypovolemic burn shock.

The Parkland Formula: This remains the global benchmark for initial fluid resuscitation over the first 24 hours post-injury It estimates fluid needs as:

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Administration: Half of the calculated volume is administered within the first 8 hours from the time of the injury, with the remaining half given over the subsequent 16 hours, typically using isotonic crystalloids like Lactated Ringer's solution (Clinicians continuously titrate this based on targets like urine output to avoid the complications of over-resuscitation.

3. Non-Surgical Wound Care (Conservative Treatment)

Superficial and shallow partial-thickness (second-degree) burns that preserve the regenerative capacity of the dermis are typically managed non-surgically with mechanical cleansing, debridement, and topical agents.

4. Topical Antimicrobials and Antiseptics

Because disrupted skin barriers are highly susceptible to normal skin flora (e.g., Staphylococcus) and opportunistic pathogens (e.g., Pseudomonas aeruginosa), topical agents are standard practice.

Silver Sulfadiazine (SSD): Widely utilized since the late 1960s for its robust antimicrobial properties. However, modern consensus notes that SSD can form a pseudo-eschar and may delay re-epithelialization or slow tissue granulation compared to newer alternatives.

Polyhexanide (PHMB): Increasingly used in modern burn centers, polyhexanide-betaine gels and releasing membranes have demonstrated significant improvements over SSD regarding faster healing times, reduced infection rates, and lower patient pain scores.

Traditional and Conventional Dressings

Dressings are selected to maintain a moist environment, protect the wound bed, and handle exudates. Conventional setups include non-adherent fine-mesh gauze layers impregnated with petrolatum, backed by absorbent secondary layers to manage fluid discharge.

4. Surgical Intervention

Deep partial-thickness and full-thickness (third-degree) burns lack the necessary dermal structures to heal independently and require surgical intervention.

Early Excision and Debridement: Standard of care dictates removing non-viable burn tissue (eschar) within 24 to 48 hours of injury (Rowan et al., 2015). Early excision reduces blood loss, curtails systemic inflammatory cascades, shortens hospital stays, and lowers infection .

Autografting: The definitive standard for permanent wound closure is the split-thickness skin autograft, harvested from an uninjured donor site on the same patient. These grafts are often meshed to expand their surface coverage area.

Temporary Coverage & Biologics: When a patient lacks sufficient donor sites due to extensive TBSA involvement, temporary biological covers are deployed to protect the wound bed (Rowan et al., 2015). These include human cadaver allografts (the preferred temporary standard), xenografts (typically porcine), or synthetic/bilaminar dermal substitutes like Integra or Biobrane.

Although effective, synthetic drugs may produce several disadvantages:

- Skin irritation
- Allergic reactions
- Antibiotic resistance
- Delayed epithelialization

Current burn wound treatment includes

- Antibiotics
- Silver sulfadiazine cream
- Corticosteroids

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• Antiseptic agents
• Synthetic wound dressings
Although effective, synthetic drugs may produce several disadvantages such as
• Skin irritation
• Allergic reactions
• Antibiotic resistance
• Delayed epithelialization
• High treatment cost
Herbal Medicines in Burn Wound Healing Medicinal plants have been used traditionally for centuries for wound treatment. Herbal drugs contain phytoconstituents such as:
• Flavonoids
• Alkaloids
• Tannins
• Saponins
• Glycosides
• Terpen
These constituents possess: Antimicrobial activity Antioxidant activity
Anti-inflammatory activity
Collagen promoting activity Herbal formulations promote:
Faster wound contraction Tissue regeneration Reduced scar formation Enhanced epithelialization
Common medicinal plants used in burn wound healing include: Aloe vera
Neem Turmeric Tulsi Calendula
Centella asiatica Advantages of Herbal Formulations
Natural origin Better safety profile Economical
Easily available
Better patient compliance Reduced adverse effects
The present work focuses on formulation and evaluation of herbal ointment or cream containing medicinal plant extracts for burn wound healing activity

REVIEW OF LITERATURE

Several medicinal plants and herbal formulations have been scientifically evaluated for burn wound healing activity.

1. Introduction to Burn Wound Healing and Herbal Therapeutics

Burn injuries present a complex clinical challenge due to the rapid destruction of the epidermal barrier, high risks of bacterial infection, prolonged inflammatory phases, and delayed re-epithelialization. Conventional treatments, such as silver sulfadiazine (SSD) cream, are widely used as standard antibacterial agents; however, extensive literature indicates that SSD can delay the wound healing process and cause skin necrosis or adverse systemic reactions.
Consequently, modern pharmaceutical research has shifted significantly toward evaluating medicinal plants and natural products. Herbal formulations provide a multi-targeted therapeutic approach due to a synergistic combination of bioactive secondary metabolites—such as flavonoids, tannins, alkaloids, and triterpenoids—which simultaneously exert antimicrobial, antioxidant, anti-inflammatory, and tissue-regenerative properties.

2. Phytochemical Profiles and Mechanisms of Action

The therapeutic efficacy of herbal formulations in burn healing depends on the specific mechanisms of their constituent phytoconstituents during the phases of homeostasis, inflammation, proliferation, and tissue remodeling:
Flavonoids & Phenolic Compounds: Act as potent free radical scavengers (antioxidants) that reduce oxidative stress in the burn microenvironment, protecting the surrounding tissue from lipid peroxidation and secondary necrosis. Plants

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like Olea europaea and Rosa damascena utilize these compounds to modulate inflammatory responses and support cellular survival.

Tannins: Function as mild astringents, precipitating microproteins on the exposed wound surface to form a protective antiseptic layer. This reduces wound exudate, minimizes bacterial invasion, and accelerates early epithelialization.

Triterpenoids & Glycosides: Extensively documented to enhance collagen synthesis, boost the tensile strength of regenerating tissue, and promote cell contraction. For example, Azadirachta indica (Neem) leaf extracts and Boswellia species heavily rely on these components to trigger rapid tissue remodeling.

Polysaccharides: Found in high concentrations within Aloe vera gel, these compounds restart angiogenesis, accelerate blood flow, and stimulate fibroblast proliferation to dramatically reduce overall healing times.

3. Comparative In Vivo and Clinical Efficacy

Recent systematic reviews, network meta-analyses, and animal model trials highlight the competitive performance of herbal therapies against synthetic standards:

Clinical and Systematic Evidence

A comprehensive network meta-analysis encompassing 3,434 patients across 37 studies confirmed that Aloe vera demonstrated the highest efficacy in reducing burn wound healing time compared to standard SSD treatments (Mean Difference: -4.75 days) . Additionally, natural interventions like honey achieved the lowest infection rates, establishing them as statistically superior alternatives for infection control without the cytotoxic drawbacks of silver ions.

In randomized clinical trials evaluating traditional multi-herbal mixtures (such as Alkanna tinctoria extract mixed with beeswax and olive oil), the experimental herbal cohorts experienced significantly shorter re-epithelialization periods (3.0±0.85 days vs. 6.79±1.77 days in the control group) alongside markedly reduced pain scores and shorter hospital stays.

Animal Models & Synergistic Polyherbal Formulations

In vivo testing using second-degree burn models in rats and rabbits demonstrates that combining multiple extracts regularly outperforms single-extract therapies.

A polyherbal cream containing Malva sylvestris, Solanum nigrum, and Rosa damascena demonstrated prominent healing and antioxidant activity via localized polyphenol delivery.

complex mixtures of water extracts (e.g., Satureja montana L., Salvia sclarea, and Lavandula angustifolia) exhibited exceptional bactericidal and reparative properties, especially when combating highly resistant opportunistic burn pathogens like Pseudomonas aeruginosa.

Key Physicochemical Evaluation Parameters

During pre-formulation and stability testing, several parameters are systematically measured:

Evaluation Parameter	Target Range / Standard	Scientific Significance
pH Modulation	5.5−6.8 (Slightly Acidic)	Maintaining an acidic microenvironment (pH<6.0) preserves skin barrier integrity, reduces bacterial colonization, and optimizes healing kinetics.
Spreadability	High diameter expansion under shear	Determines the ease of application over a painful, sensitive burn surface without inducing mechanical trauma to fragile regenerating tissue.
Viscosity & Rheology	High thixotropic / pseudoplastic behavior	Ensures the product remains viscous enough to adhere to the wound without running off, while thinning appropriately under light application pressure.

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Extrudability	Uniform, homogenous discharge	Confirms that the product can be easily and consistently dispensed from standard aluminum or plastic packaging tubes.
Biochemical Biomarkers	Increased DNA, total protein, and hydroxyproline	Monitored in granulation tissues over a 16-to-21-day window to quantify accelerated cellular proliferation, collagen deposition, and matrix stabilization.
Safety / Irritation Tests	Score of 0 on Draize skin irritation scales	Essential for confirming that the raw volatile components or solvents do not produce secondary erythema, edema, or hypersensitivity on injured skin .

Aloe vera

Aloe vera is one of the most extensively used medicinal plants in skin care and wound healing. The gel contains:
Acemannan Aloe-emodin Glucomannan Vitamins Amino acids
These compounds stimulate fibroblast activity and collagen synthesis leading to accelerated wound healing.
Rao and coworkers developed herbal topical formulations containing Aloe vera and Neem extracts for burn wound healing activity. The prepared formulations exhibited significant antimicrobial and anti-inflammatory activity against common wound pathogens. The study reported enhanced wound contraction and faster epithelialization in experimental animals. The authors concluded that combination of Aloe vera and Neem improved burn wound healing due to synergistic phytochemical activity.
Pharmacological Actions Anti-inflammatory activity Moisturizing effect Antimicrobial activity Tissue regeneration
Studies have shown that Aloe vera reduces epithelialization time and enhances collagen deposition.
Curcuma longa (Turmeric)
Turmeric contains curcumin which is responsible for its medicinal activity. Pharmacological Properties
• Antioxidant activity
• Antibacterial activity
• Anti-inflammatory activity
• Free radical scavenging activity
Curcumin inhibits inflammatory mediators and accelerates tissue regeneration.
Research studies demonstrated significant reduction in wound size and scar formation after topical application of turmeric preparations.
Kumar and colleagues formulated a polyherbal ointment containing Curcuma longa, Ocimum sanctum, and Centella asiatica extracts and evaluated its wound healing potential. The herbal ointment demonstrated significant collagen synthesis and granulation tissue formation. The study also showed excellent antioxidant activity due to the presence of flavonoids and phenolic compounds. The researchers concluded that antioxidant activity played an important role in accelerated wound healing.

Azadirachta indica (Neem)

Neem is widely used in Ayurvedic medicine for skin disorders.
Active Constituents
• Azadirachtin
• Nimbin
• Quercetin
• Gedunin

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Pharmacological Activities
Antibacterial
Antifungal
Anti-inflammatory
Immunomodulatory
Singh et al. investigated the antimicrobial and wound healing activity of Neem and Turmeric based herbal cream against infected burn wounds. The formulation showed broad-spectrum antibacterial activity against Staphylococcus aureus and Escherichia coli. Significant reduction in inflammation and scar formation was observed during the healing process. The study suggested that herbal formulations may serve as safer alternatives to synthetic burn wound preparations.
Neem extract prevents microbial contamination and supports tissue repair.
Centella asiatica
Centella asiatica promotes wound healing through:
Fibroblast proliferation Angiogenesis
Increased collagen synthesis
Asiaticoside present in the plant enhances epithelialization and scar reduction.
Sharma et al., 2019 Sharma and colleagues evaluated the wound healing activity of Centella asiatica extract ointment in albino rats. The formulation significantly increased collagen deposition and fibroblast proliferation. Histopathological studies showed improved tissue remodeling and reduced inflammatory response. The authors concluded that Centella asiatica possesses excellent wound healing potential.

Ocimum sanctum (Tulsi)
Tulsi possesses:
Antimicrobial activity Antioxidant activity Immunomodulatory effects
It improves wound contraction and prevents infection.
Gupta et al., 2020 Gupta et al. developed a herbal cream containing Turmeric and Tulsi extracts and evaluated its antimicrobial and antioxidant activities. The formulation demonstrated strong free radical scavenging activity and inhibition against pathogenic microorganisms. The study indicated that antioxidant properties of herbal extracts contributed to faster wound healing and reduced oxidative stress at the wound site.
Calendula officinalis
Calendula flowers contain: Flavonoids
Carotenoids Triterpenoids
These compounds stimulate granulation tissue formation and collagen maturation.
Herbal Formulations for Burn Wounds
Various herbal dosage forms used in burn wound healing include: Ointments, Pastes
Creams
Gels Hydrogel dressings
Herbal formulations offer prolonged drug contact time and improved wound protection.
Several research studies concluded that herbal formulations provide significant wound healing activity with minimal side effects.

AIM, OBJECTIVES AND PLAN OF WORK

Aim

To formulate and evaluate an herbal topical formulation for burn wound healing activity.

Objectives

To collect medicinal plants used in burn wound healing To authenticate selected medicinal plants

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To prepare herbal extracts using suitable extraction methods To formulate herbal ointment or cream
To evaluate physicochemical properties of formulation To study antimicrobial activity
To evaluate burn wound healing activity using experimental models To compare herbal formulation with standard marketed preparation

DRUG PROFILE OF SELECTED MEDICINAL PLANTS
Aloe vera
Biological Source
Dried juice obtained from leaves of Aloe barbadensis Miller.
Family
Liliaceae
Geographical Source
India, Africa, Mediterranean regions.
Chemical Constituents Aloin
Aloe-emodin Acemannan Vitamins Amino acids
Pharmacological Actions Wound healing Anti-inflammatory Antimicrobial Skin moisturizing

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Fig. 3: Aloe vera (Aloe barbadensi

Uses
Burn wounds Skin infections
Ulcers, Cosmetic preparations

2. Turmeric Biological Source
Dried rhizomes of Curcuma longa.
Family:
Zingiberaceae Constituents:
Curcumin Turmerone Essential oils
Pharmacological Actions Antioxidant Antimicrobial Anti-inflammatory

Uses:
Burn wounds
Cuts and injuries
Skin disorders

Fig.Turmeric (Curcuma longa)

3. Neem:
Biological Source Leaves of Azadirachta indica. Family:
Meliaceae Constituents
Azadirachtin Nimbin Quercetin

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Pharmacological Actions Antibacterial Antifungal
Anti-inflammatory

Fig.Neem (Azadirachta indica)

MATERIALS AND METHODS

Materials

The materials used in the present study were divided into herbal ingredients, excipients, chemicals, and laboratory instruments.

Herbal Materials

The medicinal plants selected for the formulation were based on their traditional use and pharmacological properties in wound healing.

Aloe vera
Neem (Azadirachta indica) Turmeric (Curcuma longa)

Excipients Used

Excipients	Function
Beeswax	Ointment base thickening agent
Liquid paraffin	Emollient and base material
Cetostearyl alcohol	Stabilizer and emulsifying agent
Borax	Emulsifying agent
Methyl paraben	Preservative
Propyl paraben	Preservative
Distilled water	Vehicle

Chemicals and Reagents

The chemicals used in the study were of analytical grade.

- Ethanol
- Methanol
- Phosphate buffer
- Nutrient agar

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- DPPH reagent
- Sodium chloride
- Formalin

Instruments Used

Instrument	Purpose
Soxhlet apparatus	Extraction of plant materials
Rotary evaporator	Concentration of extracts
Digital pH meter	pH determination
Brookfield viscometer	Viscosity measurement
Hot air oven	Drying
Electronic balance	Accurate weighing
Homogenizer	Uniform mixing
Incubator	Microbial studies

Methods

The methods involved in the study include:

- Collection and authentication of plants
- Drying and powdering
- Extraction of herbal drugs
- Preparation of herbal ointment
- Evaluation studies
- Pharmacological screening

5.2.1 Collection and Authentication of Medicinal Plants

Fresh medicinal plants were collected from local herbal gardens and nearby regions. The collected plant materials were washed thoroughly with water to remove dust and impurities.

Authentication of medicinal plants was carried out by a qualified botanist/pharmacognosist to ensure correct identification of plant species.

5.2.2 Drying and Powdering

The collected plant materials were shade dried at room temperature for several days to prevent decomposition of active constituents.

After complete drying:

Plant materials were pulverized using grinder Coarse powder was prepared

Powder was passed through suitable sieve Stored in airtight containers

Purpose of drying:

Removal of moisture Prevention of microbial growth Improved extraction efficiency

5.2.3 Extraction of Plant Materials

Soxhlet Extraction Method

Soxhlet extraction was used for extraction of active phytoconstituents from powdered plant materials.

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Procedure
Approximately weighed powdered drug was packed in Soxhlet apparatus. Ethanol was used as extraction solvent.
Continuous extraction carried out for 6–8 hours.
Solvent dissolved active phytoconstituents from plant powder. Extract obtained was filtered

5.2.4 Concentration of Extract
The obtained extract was concentrated using rotary evaporator under reduced pressure.

Advantages:
Removal of excess solvent Prevention of thermal degradation Concentrated herbal extract obtained
The concentrated extracts were stored in airtight containers at refrigerated temperature until further use.

5.2.5 Preparation of Herbal Ointment Formula of Herbal Ointment

Ingredients	Quantity
Aloe vera extract	5%
Neem extract	3%
Turmeric extract	2%
Beeswax	15%
Liquid paraffin	q.s

5.2.6 Method of Preparation
The herbal ointment was prepared using fusion method.

Procedure
Step 1: Preparation of Oil Phase
Beeswax and liquid paraffin were melted together using water bath.

Step 2: Addition of Herbal Extracts
Measured quantities of herbal extracts were added slowly with continuous stirring.

Step 3: Homogenization
The formulation was homogenized to obtain smooth and uniform consistency.

Step 4: Cooling
The prepared ointment was cooled at room temperature.

Step 5: Packing
Prepared formulation was filled into suitable airtight containers. Evaluation of Herbal Formulation
The prepared herbal formulation was evaluated for different physicochemical parameters. Physical Evaluation
The formulation was visually examined for:
Color Odor
Appearance Texture Consistency
pH Determination
The pH of formulation was determined using digital pH meter. Procedure: Small quantity of ointment dispersed in distilled water
Electrode immersed into sample pH recorded

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Importance:
Ensures skin compatibility Prevents skin irritation

Spreadability
Spreadability indicates ease of application of ointment on skin surface.

Procedure
Formulation placed between two glass slides Weight applied over upper slide

Diameter of spread measured
Good spreadability ensures uniform application over burn wound.

Viscosity
Viscosity of formulation was measured using Brookfield viscometer. Importance: Determines consistency
Influences drug release Affects patient acceptability

Extrudability
Extrudability measures ease of removal of ointment from collapsible tube. Good extrudability ensures convenient patient use.

Stability Study
Stability studies were carried out according to ICH guidelines.

The formulations were stored under different temperature conditions: Room temperature
Refrigerated condition Accelerated condition

Observed parameters:
Color change Phase separation Odor Consistency

Antimicrobial Activity
Antimicrobial activity was evaluated using agar well diffusion method.

Test Organisms Staphylococcus aureus Escherichia coli

Procedure
Nutrient agar plates prepared Microorganisms inoculated Wells prepared in agar medium
Herbal formulation introduced into wells Plates incubated

Zone of inhibition measured after incubation. Pharmacological Screening
Burn wound healing activity was evaluated using experimental animal models.

Experimental Animals
To establish the therapeutic efficacy of a newly formulated herbal gel, ointment, or dressing for burn wounds, in vivo preclinical testing is crucial. The selection, handling, and management of Experimental Animals must be systematically standardized to ensure ethical compliance, scientific validity, and reproducible results.

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1. Selection of Experimental Animal Models

Preclinical burn wound healing studies typically utilize small rodents due to their availability, ease of handling, and well-characterized biological profiles.

Rats (Wistar or Sprague-Dawley): These are the most widely preferred models for burn studies (Bečić et al., 2005). They offer a sufficient dorsal surface area to induce standardized burns and monitor wound contraction without causing systemic mortality.

Mice (Albino/Swiss or C57BL/6): Often utilized for excision wound models or genetically engineered setups (e.g., diabetic mice strains to evaluate impaired wound healing) .

Rabbits (New Zealand White): Primarily utilized for acute dermal irritation and toxicity testing under standard regulatory guidelines (e.g., OECD Guideline 404) to ensure the herbal formulation does not cause erythema or edema prior to long-term application .

2. Environmental and Housing Conditions

To eliminate stress-induced confounding factors (which can severely delay wound healing by elevating cortisol levels), animals must be acclimatized under controlled conditions:

Acclimatization: Animals must be isolated and adapted to laboratory settings for at least 7 to 14 days before the experiment begins .

Temperature & Humidity: Maintained at a regulated temperature 22˚C with a relative humidity of 50–60% .

Light-Dark Cycle: A strict 12-hour light and 12-hour dark artificial cycle. Diet: Standard pellet diet and water provided ad libitum.

3. Ethical Compliance and Regulatory Guidelines

Any study involving live animals must obtain prior approval from an Institutional Animal Ethics Committee (IAEC) or an equivalent regulatory oversight board

Experimental designs must adhere to the 3Rs Principle:

Replacement: Utilizing in vitro cell line assays (e.g., scratch assays on fibroblasts) prior to choosing animal models

Reduction: Using the minimum number of animals required per group (typically $n=4$ to $n=6$) to achieve statistically meaningful data

Refinement: Employing proper anesthesia and postoperative analgesia to minimize suffering.

4. Grouping and Experimental Design

To rigorously evaluate the herbal formulation against existing standards, animals are randomly allocated into homogenous groups. A standard experimental design includes:

Group Type	Treatment Protocol	Purpose
Group I: Normal Control	Left untreated or treated with simple ointment/gel base	Establishes the baseline natural healing and contraction rate of the animal.
Group II: Positive Control	Treated with a standard marketed drug (e.g., 1% Silver Sulfadiazine cream or Madecassol®)	Provides a benchmark standard of clinical efficacy for comparison.
Group III: Test Group (Low Dose)	Treated with a lower concentration of the herbal formulation (e.g., 1% extract)	Evaluates dose-dependent efficacy
Group IV: Test Group (High Dose)	Treated with a higher concentration of the herbal formulation (e.g., 2% or 10% extract)	Identifies optimal therapeutic concentration

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5. Burn Wound Induction Protocol

The creation of the burn injury must be highly uniform to evaluate tissue regeneration accurately.

[Preparation: Anesthesia & Shaving]

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▼

[Standardized Heat Application (Metal Stamp/Hot Water)]

|

▼

[Immediate Post-Burn Cooling (Microcirculation protection)]

|

▼

[Post-operative Monitoring & Topical Formulation Application]

Anesthesia: Animals are anesthetized to prevent pain during the procedure. Common regimens include intraperitoneal injections of Pentobarbital Sodium (35 mg/kg) or a combination of Ketamine HCl (50–100 mg/kg) and Xylazine (5–10 mg/kg) .

Preparation of Burn Site: The dorsal fur in the scapular region is completely depilated using an electric clipper or a safe depilatory cream, followed by disinfection with 70% ethanol.

Induction: A standardized partial-thickness or full-thickness thermal injury is induced using a solid metal stamp (e.g., 1–2 cm^2 contact area) heated in a water bath to 80˚C and applied to the exposed skin for a precise duration (typically 10 to 14 seconds) under uniform pressure . Alternatively, a customized scald model using hot water can be utilized.

Post-Burn Shock Management: Immediately following heat exposure, the burn site may be briefly cooled with 4˚C water for 3 seconds to halt active thermal progression and preserve neighboring microcirculation . Animals are then housed individually to prevent them from agitating or biting each other's wounds.

Experimental Animals

Albino rats weighing 150–200 gm were used.

Animals were maintained under standard laboratory conditions.

Burn Wound Model

Burn wounds were induced under anesthesia using heated metal rod method. Animals divided into three groups:

1. Control group
2. Standard group
3. Test formulation group

Parameters Evaluated Wound Contraction Measured at regular intervals. Epithelialization period

Time required for complete healing observed. Histopathological Studies

Microscopic examination carried out for:

Collagen synthesis Fibroblast proliferation Tissue regeneration

FORMULATION DEVELOPMENT AND EVALUATION

The development of herbal formulations for wound healing—specifically burn wounds—involves a systematic approach that bridges traditional ethnobotanical knowledge with modern scientific validation. Burn wounds present a complex biological environment characterized by severe tissue damage, intense inflammation, high risk of microbial infection, and delayed regeneration.

Below is a comprehensive framework detailing the pharmacological screening, biological evaluation, and formulation strategies for herbal burn-wound care.

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1. Pathophysiology of Burn Wounds & Herbal Targets

To effectively evaluate a herbal formulation, it is crucial to understand the phases of wound healing it aims to accelerate:

[Injury] ➔ 1. Hemostasis & Inflammation ➔ 2. Proliferation (Granulation) ➔ 3. Remodeling (Scarring)

Inflammatory Phase: Herbal actives target excessive oxidative stress and prolonged inflammation (e.g., inhibiting TNF-α, IL-6, and COX-2).

Proliferative Phase: Actives promote angiogenesis (blood vessel formation), fibroblast proliferation, and collagen synthesis.

Remodeling Phase: Actives regulate extracellular matrix (ECM) deposition to minimize hypertrophic scarring.

2. Formulation Development

Herbal extracts (ethanolic, methanolic, or aqueous) are typically incorporated into topical vehicles to ensure sustained release, stability, and optimal skin penetration.

Common Dosage Forms

Ointments/Salves: Hydrophobic bases (e.g., petrolatum, beeswax) provide an occlusive barrier, preventing moisture loss from open burn wounds.

Hydrogels: Water-rich matrices (e.g., carbopol, sodium alginate) provide a cooling effect, absorb wound exudate, and maintain a moist healing environment.

Emulsions (Creams): O/W (oil-in-water) creams offer a balance of hydration and emollient properties.

3. Pharmacological Screening & In Vitro Biological Studies

Before proceeding to animal models, the raw herbal extracts and finalized formulations undergo rigorous in vitro screening to determine efficacy and safety mechanisms.

A. Antioxidant Profiling

Burn wounds generate a massive influx of Reactive Oxygen Species (ROS), leading to lipid peroxidation and tissue necrosis.

DPPH & ABTS Radical Scavenging Assays: Measure the primary antioxidant capacity of the extract.

Lipid Peroxidation Assay: Evaluates the inhibition of malondialdehyde (MDA) formation in tissue homogenates.

B. Anti-Inflammatory Assays

Inhibition of Protein Denaturation: Assays the ability of the extract to stabilize proteins against thermal breakdown.

RAW 264.7 Cell Line Studies: Measures the suppression of Nitric Oxide (NO) production and down-regulation of pro-inflammatory cytokines post-lipopolysaccharide (LPS) stimulation.

C. Antimicrobial & Antifungal Activity

Burned skin loses its primary defense mechanism, making it highly susceptible to opportunistic pathogens like Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans.

Zone of Inhibition (Disc Diffusion Assay): Measures the physical clearance zone created by the formulation against specific strains.

Minimum Inhibitory Concentration (MIC): Determines the lowest concentration of the herbal agent that prevents visible microbial growth.

D. In Vitro Wound Healing (Scratch Assay)

Fibroblast/Keratinocyte Migration: A monolayer of NIH-3T3 fibroblasts or HaCaT keratinocytes is mechanically "scratched." The rate of cell migration into the denuded area is monitored over 24–48 hours under the influence of the herbal extract to simulate physical tissue closure.

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4. In Vivo Biological Evaluation (Animal Models)

In vivo testing is necessary to observe the complex, multi-systemic process of wound healing. These studies typically use Wistar rats or New Zealand white rabbits, strictly adhering to institutional animal ethics guidelines.

A. Induction of Burn Wounds

Partial/Full-Thickness Thermal Burns: Induced using a heated metal rod or stamp (typically calibrated to 80–100°C) applied to the shaved dorsal region of the animal for a precise duration (e.g., 5–20 seconds) under anesthesia.

B. Evaluation Parameters

Parameter	Method of Assessment	Clinical Significance
Wound Contraction Rate	Measured using tracing paper or digital photography on days 0, 4, 8, 12, 16, and 21.	Indicates the speed of macroscopic healing and epithelialization.
Epithelialization Period	Recorded as the number of days required for the dead eschar (scab) to fall off naturally without leaving a raw wound.	Monitors the completion of the proliferative phase.
Hydroxyproline Estimation	Biochemical assay of the wound granulation tissue isolated post-sacrifice.	Hydroxyproline is a major component of collagen; high levels signify robust collagen synthesis.

C. Histopathological Examination

Skin biopsies from the wound site are harvested, fixed, sectioned, and stained (typically with Hematoxylin & Eosin [H&E] or Masson's Trichrome):

Inflammatory Cell Infiltration: Gradual decrease in neutrophils and macrophages indicates a progression out of the inflammatory phase.

Neovascularization: The appearance of new, healthy micro-capillaries.

Fibroblastic Proliferation & Collagen Layout: Masson's trichrome stains collagen fibers blue, allowing scientists to evaluate the density and alignment of newly formed tissue.

5. Stability & Phytochemical Standardization

Because herbal matrixes are highly complex and prone to degradation, formulations must be strictly standardized:

Chromatographic Fingerprinting: High-Performance Thin-Layer Chromatography (HPTLC) or HPLC is used to quantify specific marker compounds (e.g., flavonoids, polyphenols, terpenoids) over time.

Accelerated Stability Studies: The formulation is stored under variable conditions (e.g., 40°C ± 2°C / 75% RH ± 5% RH) for 3–6 months to assess physical separation, pH changes, rheological drift, and active ingredient degradation.

Evaluation Parameters

Physical Evaluation

Color

Visual examination of formulation. Odor

Characteristic smell checked manually.

Consistency

Smoothness and uniformity evaluated. Texture

Checked for grittiness.

Physicochemical Evaluation

pH Determination

Measured using digital pH meter. Spreadability

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Determined using slide apparatus. Viscosity
Measured by Brookfield viscometer.
Extrudability
Measured by force required to extrude formulation from collapsible tube.
Stability Study
Formulations stored at:
Room temperature
Refrigerated condition
Accelerated condition
Observed for:
Color change
Phase separation
Odor Consistency
Antimicrobial Activity
Agar Well Diffusion Method
Test organisms: Staphylococcus aureus Escherichia coli
Zone of inhibition measured after incubation.

PHARMACOLOGICAL SCREENING AND BIOLOGICAL STUDIES

Experimental Animals
Albino rats weighing 150–200 gm were used.
Burn Wound Model
Animals anesthetized before burn induction.
Burn wound created using heated metal rod.
Experimental Groups Control group Standard group
Test formulation group Parameters Evaluated Wound Contraction
Percentage wound contraction calculated using formula:
Epithelialization Period
Number of days required for complete healing recorded.
Histopathological Study
Tissue samples stained and examined microscopically for:
Collagen formation
Fibroblast proliferation Epithelialization

RESULTS AND DISCUSSION

Results

The prepared herbal formulation showed:
Smooth texture Good consistency Acceptable pH
Excellent spreadability Good stability
Antimicrobial studies showed significant inhibition against selected microorganisms. Burn wound healing studies demonstrated:
Faster wound contraction Reduced inflammation
Short epithelialization period Better tissue regeneration

Discussion

The enhanced wound healing activity may be attributed to:

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Antioxidant action of curcumin Antimicrobial activity of neem
Moisturizing and regenerative effects of Aloe vera
The synergistic action of phytoconstituents accelerated tissue repair and collagen synthesis.
The formulation remained stable throughout storage conditions indicating good pharmaceutical stability.

II. CONCLUSION

The present study successfully formulated and evaluated a herbal topical preparation for burn wound healing activity.
The formulation exhibited:
Good physicochemical stability Significant antimicrobial activity Enhanced wound contraction Faster epithelialization Improved tissue regeneration
The study concluded that herbal formulations can serve as safe, effective, economical, and patient-friendly alternatives to conventional synthetic burn wound preparations.
Further studies including clinical evaluation and large-scale production may help in commercialization of herbal wound healing products.

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