Formulation and Evaluation of Wound Healing Cream Using Extracts of Azadirachta Indica and Tridax Procumbens

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ABSTRACT
Wound healing is a cellular and biochemical process of restoring normal structure functions of damaged tissue. Healing is a natural phenomenon by which body itself overcome the damaged to the tissue but the rate of healing is veritably slow and chance of microbial infection is high. enhancement in healing process can be negotiate either the time needed for mending or to minimize the uninvited consequences. India has a rich tradition of herbs predicated knowledge on healthcare system. Several medicinal herbs proved to be a wound healer were related and formulated for treatment and direction of injuries. Various herbal products have been used in operation and treatment of injuries over the times. The present research attempt to emphasizes on formulation of a wound healing dosage form using herbal drug extracts of Azadirachta indica and Tridax procumbens.

Keywords: Azadirachta Indica, Tridax Procumbens, Herbal Cream, Traditional Herbs.

INTRODUCTION:
Skin Anatomy: The skin is the largest organ of the body, counting for about 15 of the total adult body weights. It performs numerous vital functions, including protection against external physical, chemical, and birth assailers, as well as precluding of redundant water loss from the body and a part in thermoregulation. The skin is composed of three layers the epidermis, the dermis, and subcutaneous cell (Kanitakis, 2002). The epidermis, consists of a specific constellation of cells known as keratinocytes, which serve to synthesize keratin, a long, threadlike protein with a defensive function. The middle status, the dermis, is unnaturally made up of the fibrillar structural protein known as collagen. The dermis lies on the subcutaneous cell, or panniculus, which contains small lobes of fat cells known as lipocytes. Thick status of skin has an epidermis consistence of 0.07-0.15 mm. Thick, non-air-bearing(rough) set up on palmar and plantar fase has no hair, sebaceous gland. Thinner skin is set up over the rest of body, but is especially thin over the eyelids and it composed of lower cellular layers. [¹¹]
DERMIS:
The order of skin set up between the epidermis and hypodermis (subcutaneous cell) is the dermis. The dermis varies in consistence from 0.3 mm on eyelid to 3.0 mm on the reverse, making it much thicker than the epidermis. Blood vessels and nervous course through the dermis furnishing both nutrition and sensation. Various adjuncts including sweat glands, hair follicles, and sebaceous glands can also be set up in this place. The dermis provides cell nutritive and structural support to the epidermis. [2]

HYPODERMIS:
The hypodermis begins beneath, the dermis and above the muscle and composed mainly of adipocytes, serves a variety of functions in the body. It provides insulation from cold, cushions deep cell from violent trauma, provides buoyancy, is a respiratory for energy, and indeed acts as an endocrine organ. Adipocytes contain fat lobules that are separated by stringy septa composed of collagen and large blood vessels. This collagen is continued with the collagen set up in the dermis.

Functions of skin:
1. Thermoglutation
2. Protection:
3. Cutaneous Sensation:
4. Excretion and Absorption:
5. Synthesis of Vitamin D

Pathophysiology of Wound Healing: Wound healing is a complex, dynamic process supported by a myriad of cellular events that must be tightly coordinated to efficiently repair damaged cell. Derangement in wound- linked cellular behaviours, as occurs with diabetes and ageing, can lead to healing impairment and the conformation of habitual, non-healing injuries. These wounds are a significant socioeconomic burden due to their high frequency and rush. therefore, there's a critical demand for the bettered natural and clinical understanding of the mechanisms that underpin wound repair. The skin has also evolved effective and rapid-fire mechanisms to close breaches to its wall in a process inclusively known as the wound healing response. Wound form is classically simplified into four main phases Haemostasis, Inflammation, Proliferation and Dermal remodelling, which affect in architectural and physiological restoration.

1. Haemostasis: Incontinently after injury, damaged blood vessels rapid-fire contract and a blood clot forms precluding exsanguination from vascular damage. Platelets, principle contributors to haemostasis and coagulation, are actuated when they encounter the vascular subendothelial matrix. Platelet receptors (e.g. glycoprotein) interact with extracellular matrix proteins (e.g. fibronectin, collagen and von Willebrand factor), promoting adherence to the blood vessel wall. Platelets clump together at the injury point to produce a fibrin clot a many second just after the injury.

2. Inflammation: Inflammation evolved as the primary defence against pathogenic crack irruption. This vulnerable response is initiated by injury convinced signals; damage- associated molecular patterns (DAMPs) released by necrotic cells and damaged towel, and pathogen associated molecular patterns (PAMPs) from bacterial factors. This phase begins incontinently after the crack and generally lasts for about 24 and 48 hours and may continue for about two weeks.

3. Proliferation: This is the alternate phase of wound healing and generally persists up to two days to twenty- one days after the first phase. Angiogenesis, collagen installation, granulation tissue growth, epithelialization, and wound shrinking are all characteristics of this phase.
4. **Remodelling**: Remodelling of the ECM spans the entire injury response, beginning with the original deposit of a fibrin clot, and ending several times latterly with the arrangement of a mature, type I collagen-rich scar. Fibroblasts are the major cell type responsible for wound ECM remodelling, replacing the original fibrin clot with hyaluronan, fibronectin and proteoglycans, and forming mature collagen fibrils latterly in shape. [4]

**Side effects include of synthetic drugs:**
- Skin ulceration
- Ulcer infection
- Hypersensitivity disorder
- Tunnelling of ulcer (a wound that has progressed to form passageways underneath the surface of the skin)
- Erythema (superficial reddening of the skin, as a result of injury or irritation causing dilatation of the blood capillaries) [5]

Recent trends and current request analysis suggest demand for herbal substance, including herbal creams, is accelerating over synthetic creams due to their lower toxin and smaller side personal effects, followed by further skin sustenance capability, according to several published studies. Also, published literature lacks any modernized, collected explicatory review on herbal creams. Thus, we designed the present communication to carry out the current demand. The herbal creams contain substantially natural substances uprooted from herbal manufactories that have health and nutritive benefits without poisonous or adverse things. The cream bases that have been used to formulate the creams are also attained from herbal manufactories also, the published literature substantially focuses on various aspects of cream substance only and still lacks any collected explicatory review on herbal creams. [6] The herbal drugs contain a lot of different composites which some of them have great complications. Manufactories substance similar as polysaccharides, mucilage’s and tannins may modulate and modify the goods of active factors. It has been shown that the whole herbal extracts cannot be mimicked by administering purified and segregated ingredients of the flavourings. [7]

Creams serve a variety of cosmetic functions, including cleansing, beautifying, enhancing appearance, protecting, and medicinal. These Topical formulations are utilized to deliver drugs locally, either into the mucous membrane or the skin’s underlying layer. These treatments are intended to be applied topically to improve the drug’s site-specific delivery to the skin for skin conditions. Since creams are made using methods developed in the pharmaceutical business, they are regarded as pharmaceutical products. Both medicated and unmedicated creams are widely used to treat dermatoses and other skin problems. Based on phases, creams can be categorized as either w/o or o/w types of emulsion. Traditionally, semisolid formulations that are either water-in-oil (such as cold cream) or oil-in-water (such as vanishing cream) have been referred to as "cream.

**Classification of Cream:**

**A. All the skin creams can be classified on different basis:**
1. According to function: Example- Purification, foundation, massage etc.
2. According to characteristic properties: Example- Cold cream, vanishing cream.
3. According to nature or type of emulsion.

**B. Types of creams according to function, characterization property, types of emulsion**

Make up creams:

Foundation cream
Vanishing cream

- Cleansing lotion, cleansing cream (w/o emulsion)
- Winter cream (w/o emulsion): Cold cream, Moisturizing cream
- All-purpose cream or general creams
- Night cream or Massage cream
- Skin protective cream
- Hand and Body cream

Advantages of Cream:

- Negations of First-pass metabolism
- Accessible and easy to apply.
- It does not show any side effects on other body organs.
- Avoid changes of medicine situation on inter and intra patent variations

Disadvantages of Cream:

- Skin irritation and some medicines show low penetration through skin.
- Possibilities of allergic reaction.
- Small plasma immersion.
- Larger particle size medicine is showing the poor effect.

Table No.1: Possible Herbal drugs with significant findings for Wound Healing Property

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Common Name</th>
<th>Biological Name</th>
<th>Family</th>
<th>Possible Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Manjistha</td>
<td>Rubia Cordifolia Linn</td>
<td>Rubiaceae</td>
<td>Blood purifier, Immunomodulator, anti-inflammatory and antioxidant</td>
</tr>
<tr>
<td>2.</td>
<td>Kapura Tulasi</td>
<td>Ocimum kilimandscharicum</td>
<td>Laminaceae</td>
<td>Cough, bronchitis, viral infections, anorexia and also wound healing</td>
</tr>
<tr>
<td>3.</td>
<td>Sarwa Wranvishapaka</td>
<td>Tephrosia purpurea Linn</td>
<td>Leguminaceae</td>
<td>cures diseases of kidney, liver, spleen, heart and blood. The dried herb is tonic, laxative, diuretic</td>
</tr>
<tr>
<td>4.</td>
<td>Sausage Tree</td>
<td>Kigelia pinnata</td>
<td>Bignoniaceae</td>
<td>anti-amoebic, antifungal, antiulcer, antibacterial, shows significant wound healing activity</td>
</tr>
<tr>
<td>5.</td>
<td>Chia, Pignut</td>
<td>Hyptis suaveolens Linn</td>
<td>Lamiaceae</td>
<td>anti-inflammatory anti-fertility</td>
</tr>
<tr>
<td>6.</td>
<td>Teak, Sagwan</td>
<td>Tectona grandis Linn</td>
<td>Verbenaceae</td>
<td>Injuries like burn, inflicted wound and skin ulcers.</td>
</tr>
<tr>
<td>7.</td>
<td>Gorakhmuni</td>
<td>Sphaeranthus indicus Linn</td>
<td>Asteraceae</td>
<td>Odema, arthritis, filariasis, gout and cervical adenopathy</td>
</tr>
<tr>
<td>8.</td>
<td>Papaya</td>
<td>Carica papaya Linn</td>
<td>Caricaeae</td>
<td>Fruits possess wound healing properties; papaya latex was applied to the burn wound</td>
</tr>
</tbody>
</table>
9. Gokshur or Gokharu | *Tribulus terrestris* | Zygophyllaceae | Diuretic, anthelmintic, cytotoxic, Anti-microbial, Anti-fungal

10. Eyilik | *Arnebia densiflora* | Boraginaceae | Roots of this plant have been reported for Wound closure and collagen production were faster

11. Mengkudu besar, Indian Mulberry | *Morinda citrifolia* | Rubiaceae | Arthritis, atherosclerosis, bladder infections, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness

12. Napoleon’s Hat | *Napoleona imperialis* | Lecythidaceae | Analgesic, tonic, anti-tussive, anti-asthmatic, and wound dressing

13. Vinca Rosea | *Catharanthus roseus* | Apocyanaceae | Anti-neoplastic agents to treat leukaemia, Hodgkin’s disease, 

14. Drumstick tree | *Moringa oleifera* | Moringaceae | anti-tumour, hypotensive, antioxidant, anti-inflammatory

15. Turmeric | *Curcuma longa* | Zingiberaceae | antibacterial, anti-fungal and anti-inflammatory activities

16. Tulsi | *Ocimum sanctum* | Labiatae | For skin diseases, hepatic disorders and as an antidote for snake bite

17. Coat Button | *Tridax procumbens* | Asteraceae | Juice of leaves for bleeding from cuts and bruises in animals

18. Sunflower | *Helianthus annus* | Asteraceae | Inflammation of eyes, sores, dysuria, colic, tiger bites and bone fractures

19. Garden Cress | *Lepidium sativum* | Cruciferae | Source of vitamins, diuresis effect, a stimulant of bile function, and a cough reliever

20. Lanata | *Lanata camara* | Verbenaceae | Abortifacient, antimalarial, anti-inflammatory crack healing functions

**Materials and methods:**

**Table No 2: Drug profile** \(^{27,28}\)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Common Name</th>
<th>Biological Name</th>
<th>Family</th>
<th>Chemical Constituents</th>
<th>Medicinal Uses</th>
<th>Treating Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Neem</td>
<td><em>Azadirachta indica</em></td>
<td>Maliaeceae</td>
<td>Azadirachitin, salannin, nimbasterol, oleic</td>
<td>Wound healing, treat acne, treat fungal infection</td>
<td>Wound healing, Antiulcer effect,</td>
</tr>
</tbody>
</table>

\(^{27,28}\)
acid and stearic acid.

<table>
<thead>
<tr>
<th>Tridax</th>
<th>Tridax Procumbens</th>
<th>Asteraceae</th>
<th>Alkaloids, steroids, flavonoids</th>
<th>wound healing, anti-inflammatory</th>
<th>Antimicrobial activity</th>
<th>Anti-diabetic activity</th>
</tr>
</thead>
</table>

Collection and Authentication of plant material: Both the plant material was collected from local area. These plant materials are authenticated by D.B.F Dayanand college of Arts and Science, Solapur.

Method of Extraction:

- Maceration: In this procedure, the solid plant parts are put in a stoppered container with the entire solvent and let to stand, often agitated, for at least three days (or up to seven days), until the soluble matter dissolves. Following a period of standing, the mixture is strained (with sieves or nets), the marc is crushed, and the combined liquids are clarified (cleaned by filtration) or by decantation. To stop microorganisms from growing when the solvent is water and the maceration process is prolonged, a tiny amount of alcohol may be added.[32]

FORMULATION:

Preparation of Herbal Extract:
One of the most basic extraction methods is maceration, which involves soaking coarsely ground plant material in solvents including methanol, ethanol, ethyl acetate, and acetone and then spinning it for a while on a magnetic stirrer to extract the component. To get rid of dust and other unwanted objects, water was used to wash the leaves of both plant samples. After being cut off from the stems, the leaves were dried for two days at 40 to 45°C. The leaves were pulverized in a grinder once they had dried. The 50g powdered sample was macerated for three days in 100ml of ethanol solvent to extract the material. Following extraction, the sample underwent filtering. The solvent made of methanol evaporated.
Figure 6: Process of Extraction

- **Preparation of o/w emulsion cream**: In a single beaker, the emulsifier and the oil-soluble ingredients are melted in a water bath at 75°C. Preservatives and water-soluble ingredients are also taken and melted at 75°C in a different beaker of water. Following heating, the water phase was gradually added to the oil phase in a mortar and pestle, and the mixture was triturated until a clicking sound was produced. Finally, preservatives and/or fragrances are applied when the temperature drops. There will be more water in this preparation than oil. [33]

<table>
<thead>
<tr>
<th>Table No 3: Formulation Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr No</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
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<td>3.</td>
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<td>4.</td>
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<td>5.</td>
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<tr>
<td>6.</td>
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<tr>
<td>7.</td>
</tr>
<tr>
<td>8.</td>
</tr>
</tbody>
</table>

**Evaluation Parameter of developed Formulation:**

1. **Physical Appearance**: The Physical appearance of the cream can be observed by its dye, harshness and graded.

2. **Spreadability**: Acceptable quantity of sample is taken between two glass slides and a load of 100gm is applied on the slides for 5 twinkles. Spreadability can be expressed as

\[ S = \frac{m \times l}{t} \]

Where,

- \( m \) = weight applied to upper slide = 100
- \( l \) = length moved on the glass slide = 4.5
T = time taken = 5 sec

\[
\frac{100 \times 4.5}{5} = 90
\]

1. **Homogenesity:** The formulation was tried for the uniformity by visual appearance and by touch.
2. **Removal:** The ease of disposal of the creams applied was examined by washing the applied part with valve water.
3. **Dye test:** The scarlet colour is mixed with the cream. set a drop of cream in a slide and lid with a cover slip and examine it under a microscope. If the scatter drop appears red and the ground colourless also it's o/w type and the back condition appears in w/o type of creams.
4. **After feel:** Emollience, slipperiness and quantity of residue left after the operation of fixed quantity of cream was checked.
5. **Smear:** After use of cream, the nature of film or smear found on the skin were examined.
6. **Washability:** Formulation was applied on the skin and also ease prolong of washing with water and checked.
7. **Stability study:** Physical stability study tests of the formulation were carried out for first day, after three month and six months at temperature 37°C for various physicochemical parameters. The formulation was found to be physically stable at different physicochemical parameter for six months.

**RESULTS:**

Figure 7: Paper Chromatography for confirmation of *Nimbin* and *Azadirachtin*

Figure 8: Spreadibility
Phytochemical Screening:
The extract was studied for its phytochemical analysis by qualitative chemical test. The presence of major phytoconstituents were present in following table.

Phytochemical analysis for the prepared extracts:

- **Confirmatory Tests for Azadirachta indica**
  1. **Test for Triterpenoids**: Two ml of trichloroacetic acid was added to 1ml of extract. The presence of terpenoids was confirmed by the formation of red precipitate.
  2. **Test for Tannin**: 0.5gm of plant extract and 2ml of water then heat on water bath, filter add 1ml 10% FeCl₃. Formation of blue-black solution.
  3. **Test for Flavonoids**  
     5ml distilled water + 0.2 gm extract mixed add 1ml of 1% AlCl₃. Formation of light yellow ppt
  4. **Test for Amino Acid**  
     0.2 gm extract + 5ml distilled water mixed left for 3 hr then filter,2ml filtrate + 0.1ml million reagent. Formation of yellow.

- **Confirmatory Tests for Tridax procumbens**:
  1. **Test for Alkaloids**  
     3ml conc. Extract + 1ml HCL heat for 20 min cooled and filter. Filtrate treated with wagoner reagent. Formation of brown reddish ppt.
  2. **Test for Phenols**  
     Test extract+4 drops alc. FeCl₃ sol. Formation of bluish black colour.
  3. **Test for Flavonoids**  
     Extract + 10% NaOH. Formation of intense yellow colour \[26\]

- **Confirmation of Phytoconstituents by Paper Chromatography Method**:
  1. **Selection of filter paper**: Selection of filter paper is based on the sample quality.
  2. **Preparation of solution**: Solution is prepared by adding similar quantity of solvents like Ethanol and Acetic Acid in equal ratio.

**Spotting of Extract:**
The cotton plug was submerged in the center of the paper, and the extract was visible around the borders of the plug. Added the paper and sample to a petri dish. The extract point is covered by the solvent as it permeates the paper. Depending on how soluble they are, the constituents flow with the solvent. As seen, the separated components show up as distinct spots on the solvent's course. The spotting of Nimbin and Azadirachtin is distributed in the following figure no 7.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Phytochemical constituents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Triterpenoids</td>
<td>Present</td>
</tr>
<tr>
<td>2.</td>
<td>Tannin</td>
<td>Present</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>4.</td>
<td>Amino acid</td>
<td>Present</td>
</tr>
</tbody>
</table>
Test for *Tridax procumbens*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemical Constituents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Phenol</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

According to the obtained results the confirmation of Nimbin and Azadirachtin from the neem leaf extract is carried out by (method of Maceration) performing Paper Chromatography Method. The ethanolic extract of neem leaves did not cause damage to the skin. This is due to the active compound of the ethanol extract of neem leaves which is an antioxidant. The neem leaf ethanol extract contains flavonoids, alkaloids, tannins, saponins, and steroids. The active compounds that act as antioxidants are flavonoids. Mallick (*et al.*) reported that the methanolic extract of the neem leaf had no toxic effect on the rat even at high doses.

**CONCLUSION:**

In Ayurveda *Tridax procumbens* and Neem were used for their various medicinal properties like antibacterial, antifungal, anti-inflammatory, wound healing. Thus, these formulations could become a medium to use these medicinal properties effectively and easily as a formulation’s dosage form like ointments and cream using locally available plants. Based on antimicrobial efficacy, two different local plants were taken and their ethanolic extracts were incorporated in the most effective ratio inappropriate base. The phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, sterols, saponins, proteins, and other miscellaneous phenolic components are believed to play a vital role in the healing of the wound by significantly increasing the rate of wound closure and epithelisation. The formulation developed from *tridax* and neem showed significant results so it can be further used commercially to develop wound healing cream.

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