

Formulation and Evaluation of Polyherbal Ointment for Wound Healing and Anti-Microbial Activity

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Abstract: *AIM: Formulation And Evaluation Of Poly Herbal Ointment For Wound Healing And Anti-Microbial Activity*

Objective: Any physical harm involving tearing, cutting, or puncturing the skin is called a wound. Numerous medications derived from plants have been shown to speed up the healing of various wound types. The goal of the current study was to create a herbal formulation with herbs that have been shown to improve cell migration and proliferation as well as reduce infection and inflammation and speed up the healing process. The use of herbal medicines and interest in them has grown significantly in recent years, even in places with access to contemporary medical care. Because medicinal plants are the richest source of plant-derived compounds and herbal medicines, there has been a recent surge in interest in these products due to their wide range of applications.

MATERIAL AND METHOD: The goal of this work is to manufacture and assess an ointment made of extracts from turmeric (Curcuma longa) and neem (Azadirachta indica). The maceration process was utilized to create the ethanolic extracts. After preparing the ointment base, the extract was incorporated into it using the levigation procedure to formulate the ointment. Its physicochemical properties, such as color, odor, pH, spreadability, extrudability, consistency, diffusion studies, solubility, and washability, were assessed once the formulation was finished. Additionally, the formulation's stability at different temperature circumstances was assessed, and the results of the diffusion, spreadability, and irritancy studies remain unchanged. Thus, it might develop into a medium for utilizing the therapeutic qualities of turmeric and neem in an easy-to-use dose form.

Keywords: Calophyl luminophylum, Dodonaea, Azadirachta indica, wound healing.

I. INTRODUCTION

WOUND HEALING

This physical damage involves tearing, cutting, or puncturing of the skin. Whenever it is exposed to air, microorganisms are entered the wound, which leads to infection. The wound may be defined as a loss or breaking of cellular and anatomical or functional continuity of living tissue [1].

The typical wound-healing response is a coordinated series of processes that start with the damage. The healing cascade is activated when platelets come into contact with exposed collagen leading to platelet aggregation and there release of clotting factors result in the deposition of a fibrin clot at the site of injury. The fibrin clot acts as a temporary matrix and prepares the body for the healing processes that follow. At the site of injury, inflammatory cells also accompany platelets and deliver important signals called cytokines or growth factors. The fibroblast is the connective tissue responsible for collagen deposition that is needed to repair the tissue injury. In normal tissues, collagen provides strength, integrity, and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and restore an atomic structure and function. In the present work formulations were prepared to accelerate wound healing process [2].

II. ANTI-MICROBIAL

The word Anti-microbial was derived from the Greek words Anti (against), macros (little) and bios (life) and refers to all agents that act against microbial organisms. The introduction of Anti-microbial agents on the surface of skin[3]. The skin has three layers. Beneath the surface of the skin are nerves, nerve ending, glands, hair follicles and blood vessels. A typical human skin surface is known to include, on the normal 40-70 hair follicles and 200-300 sweat ducts on every square centimeter of the skin [4]

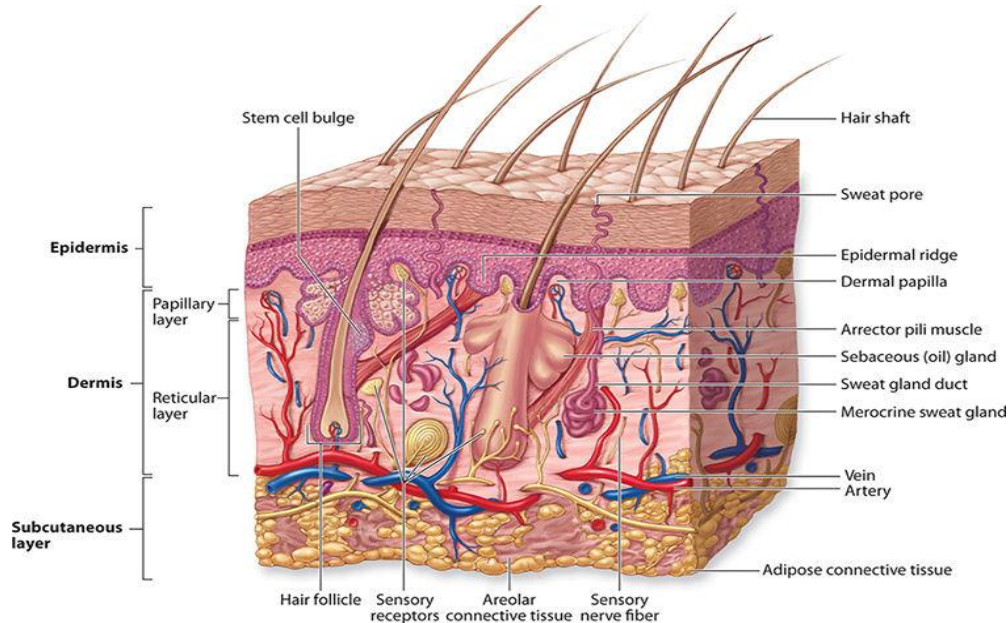


Fig.no 1 PHYSIOLOGY OF HUMAN SKIN

III. TOPICAL DRUG DELIVERY

Advantages of topical drug delivery system:

- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Avoid of risk.
- Drawbacks of intravenous treatment as well as the various absorption circumstances, such as pH variations, the presence of enzymes, gastric emptying time, etc.
- Continuous drug input leads to efficacy with a reduced total daily dosage of the drug.
- Prevent changes in medication levels between and within patents.
- The medication or its excipients may cause skin irritation or mastitis.
- It is only appropriate for medications whose actions depend on extremely low plasma concentrations.
- Applicable exclusively to medications whose actions depend on extremely low plasma concentrations[5]

EPIDERMIS

The stratified keratinized squamous epithelium that makes up the epidermis, the topmost layer of skin, varies in thickness depending on the area of the body. The palms of hands and the soles of feet have the highest concentration of it. The dermis's interstitial fluid, which supplies nutrients and oxygen and drains away as lymph, bathes the epidermis's deeper layers. The epidermis lacks blood vessels and nerve endings[5]

DERMIS

The dermis is elastic and hardy. It is made of connective tissue, and the matrix is made up of collagen and elastic fibers entwined. Stretch marks, also known as persistent striae, are a result of the rupture of elastic fibers in the skin, which can happen during pregnancy or after obesity. Wrinkles appear as a result of the aging process because collagen fibers

lose their capacity to bind water and provide the skin its tensile strength. The cells that make up the dermis are called mast cells, fibroblasts, and macrophages. A variety of levels of adipose (fat) tissue and areolar tissue are present under the skin's deepest layer [5].

IV. SUBCUTANEOUS GLANDS

Subcutaneous glands, situated within the deepest layer of the skin known as the subcutaneous tissue, comprise sebaceous and sweat glands, each serving crucial roles in skin physiology. Sebaceous glands produce sebum, an oily substance that lubricates skin and hair, preventing dryness and defending against microbial infections. Sweat glands, including eccrine and apocrine types, regulate body temperature by secreting sweat, which cools the body through evaporation. Eccrine glands, distributed across the body, produce a watery sweat primarily composed of water and salts. Apocrine glands, found in areas like the armpits and genital region, produce a thicker sweat containing proteins and lipids that, when broken down by skin bacteria, can cause odor. These glands also contribute to skin immunity by producing antimicrobial substances. Disorders affecting subcutaneous glands, such as acne or hyperhidrosis, highlight their significant impact on skin health and overall well-being. Understanding their functions aids in managing and treating related dermatological conditions effectively[6].

HERBAL PLANTS USED FOR WOUND HEALING AND ANTI-MICROBIAL ACTIVITY

NEEM (*AZADIRACHTA INDICA*)

Azadirachtaindica, commonly known as neemornim tree. It belongs to the family meliaceae .It is typically grown in tropical and semi-tropical regions^[7]Useful in eczema,ringwormand scabies. Wound healing,Anti-Inflammatory,Anti-bacterial Anti-septic,Anti-fungal^[7].

Fig.no.1 NEEM



Kingdom	Plantae
Subkingdom	<i>Tracheobionta</i>
Division	<i>Magnoliophyta</i>
Class	<i>Magnoliopsida</i>
Subclass	<i>Rosidae</i>
Order	<i>Sapindales</i>
Family	<i>Meliaceae</i>
Genus	<i>Azadirachta</i>
Species	<i>Indica</i>

Table.no.1. Scientific Classification:

TURMERIC (*CURCUMALONGA*)

Curcuma longaisa flowering plant of the ginger family zingiberaceae. It requires temperature between 20 to 30 degree Celsius. It is native to the Indian sub continent and South east Asia^[7]. Medicinal uses: wound healing, Anti-oxidant, Alzheimer's disease, Anti-cancer. It is used as Anti-septic, expectorant, condiment or spice. It is rich in Anti-oxidants, Anti-microbial, Anti-inflammatory and wound healing property, research conducted has demonstrated uses of turmeric in the treatment of Arthritis, liver disease^[7].

Fig.no.2.turmeric



Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	<i>Curcuma</i>
Species	<i>longa</i>

Table.no.2 Scientific Classification

ALOE VERA (*ALOE BARBADENSIS*):

It is a complex plant containing biologically active substance.

Medicinal uses: Wound healing, Heart burn relief, Lower blood sugar, inflammation reduction^[7].



Fig.no.3.aloe vera

Kingdom	Plantae
Subkingdom	<i>Tracheobionta</i>
Division	<i>Magnoliophyta</i>
Class	<i>Liliopsida</i>
Subclass	<i>Liliidae</i>
Order	<i>Liliales</i>
Family	<i>Aloaceae</i>
Genus	<i>AloeL</i>
Species	<i>Aloeverb(L)</i>

Table.no.3 Scientific Classification

V. COLLECTION METHOD OF MATERIAL⁽⁸⁾

5.1.Extraction of Neem Leaves

1 Collection and Washing :

Gather fresh leaves of *Azadirachtaindica* (Neem) and wash them thoroughly with distilled water to remove any dirt or impurities.

2. Drying

Spread the washed leaves in a shaded area and allow them to dry for 10 days. This drying period helps remove moisture and preserves the active compounds in the leaves.

3. Grinding :

Once dried, grind the leaves into a fine powder using a suitable grinder or mortar and pestle.

4. Ethanol Imbibition :

Take 100 grams of the powdered Neem leaves and imbibe them with 350 ml of 90% ethanol (ethyl alcohol). Allow the mixture to stand for 3 hours to facilitate the initial extraction process.

5. Maceration :

Transfer the imbibed mixture (Neem leaf powder + ethanol) into a percolator setup.

Add an additional 150 ml of 90% ethanol to the percolator.

Macerate the mixture for 7 days, ensuring occasional stirring or agitation during this period. Maceration allows for thorough extraction of active compounds into the solvent.

6. Collection of Extract :

After 7 days of maceration, collect the ethanolic extract from the percolator.

The extract obtained will have a blackish-green color due to the presence of Neem leaf compounds dissolved in ethanol.

7. Concentration:

Concentrate the collected extract using a suitable method (e.g., rotary evaporator or gentle heating under reduced pressure) until a blackish-green residue is obtained.

This concentrated extract contains the active constituents of Neem leaves in a higher concentration.

8. Storage :

Store the concentrated ethanolic extract in an airtight container.

Keep the container in a cool, dark place to protect the extract from light and heat, which can degrade its potency over time.

5.2. Extraction of Turmeric Rhizomes

1. Preparation :

Take dried rhizomes of *Curcuma longa* (Turmeric) and grind them into a fine powder, similar to the process used for Neem leaves.

2. Extraction Process :

Follow the same extraction procedure as described for Neem leaves:

Imbibe 100 grams of Turmeric rhizome powder with 350 ml of 90% ethanol.

Allow to stand for 3 hours.

Transfer to a percolator and add an additional 150 ml of 90% ethanol for maceration.

Macerate for 7 days with occasional stirring.

3. Collection and Storage :

After maceration, collect the ethanolic extract from the percolator.

The extract obtained from Turmeric rhizomes will have a crimson red color due to the presence of curcuminoids dissolved in ethanol.

Store the Turmeric extract in an airtight container, similarly in a cool and dark place to preserve its color and bioactive compounds.

VI. EXPERIMENT WORK

6.1 INGREDIENTS AND QUANTITY FOR 10 GM

Sr.No	Ingredient	Qty	Medicinal use
1)	Bees wax	2 gm	Blending agent
2)	Coconut oil	2 gm	Stabilizer
3)	Petroleum jelly	2 gm	Emollient
4)	Lanolin	0.5 gm	Emulsifying agent
5)	(SLS) Sodium lauryl sulphate	0.10 gm	Solubilising agent, Emulsifying agent
6)	Methyl paraben	0.10 gm	Anti-bacterial Agent,Preservatives

TABLE: 6.1 FORMULATION OF OINTMENT

Sr.no	Ingredient	Biological Name	Qty	Part used
1)	Prepared Neem extract	<i>Azadirachta indica</i>	2 gm	Leaves
2)	Prepared turmeric extract	<i>Curcuma longa</i>	0.5 gm	Rhizomes
3)	Aloe Vera	<i>Aloe barbadensis</i>	1 gm	Aloe Vera gel

TABLE:6.2 FORMULATION OF HERBAL OINTMENT

Procedure:

Step 1: Preparation of Herbal Extracts

1. Neem Extract :

Grind neem leaves finely using a mortar and pestle.

Boil the ground neem leaves in water (1:5 ratio of leaves to water) for 20-30 minutes.

Let it cool, then strain to obtain the neem extract.

2. Turmeric Extract :

Mix turmeric powder with a small amount of ethanol (or water, if ethanol is not available) to form a paste.

Stir well until the turmeric is completely dissolved to create the turmeric extract.

3. Aloe Vera Extract :

Cut fresh aloe vera leaves and extract the gel using a spoon.

Blend the gel to obtain a smooth consistency, creating the aloe vera extract.

Step 2: Preparation of Ointment Base

1. Melt the Waxes and Oils :

Weigh out beeswax, coconut oil, petroleum jelly, and lanolin according to your formulation (2 gm beeswax, 2 gm coconut oil, 2gm petroleum jelly, 0.5 gm lanolin).

Place these ingredients in a clean, heat-resistant container (glass beaker or stainless steel bowl).

2. Heat and Stir :

Use a double boiler setup water bath to melt the mixture gently until all ingredients are fully liquefied.

Stir with a stirring rod to ensure uniform heating and mixing.

3. Incorporate Active Ingredients :

Once melted, add the herbal extracts (neem, turmeric, and aloe vera extracts) into the melted mixture.

Stir continuously to ensure the extracts are evenly distributed throughout the ointment base.

4. Add Preservatives :

Add a small amount of sodium lauryl sulfate (SLS) and methylparaben into the mixture for stability and preservation.

Stir well to ensure the preservatives are thoroughly mixed.

Step 3: Cooling and Final Preparation

1. Cooling Process :

Allow the mixture to cool at room temperature, stirring occasionally to prevent separation of ingredients.

2. Packaging :

Once the ointment reaches a semi-solid consistency, pour it into clean, sterilized containers suitable for ointments.

Allow it to cool and solidify completely before sealing the containers.

3. Labeling and Storage :

Label each container with the ointment name, list of ingredients, and usage instructions (Apply topically to clean wounds as needed).

Store the ointment in a cool, dry place away from direct sunlight to maintain its stability.

VII. RESULT AND DISSCUSION

EVALUTION TEST

Physical Properties[8]

Colour and Odour Examination

Objective: Assess the visual appearance and smell of the ointment.

Colour: Examine the ointment visually under appropriate lighting conditions to describe its colour.

Odour : Smell the ointment to characterize its odour and note any distinct aromas.

Organoleptic Parameters[9]

Appearance, Texture, and Consistency

Objective: Evaluate the sensory attributes affecting consumer perception.

Appearance: Assess the overall look of the ointment, noting any deviations or desired characteristics.

Texture: Determine texture by evaluating the smoothness or grittiness of the ointment.

Consistency: Judge the uniformity and thickness of the ointment.

pH Measurement[9]

Objective: Determine the acidity or alkalinity of the ointment.

Prepare a solution of the ointment by mixing with distilled water (typically 100 ml of water).

Allow the solution to stabilize for 2 hours. Measure the pH using a digital pH meter calibrated for accuracy.

Spreadability[9]

Objective: Measure how easily the ointment spreads on the skin.

Place a defined amount of ointment sample between two glass slides.

Apply a specific weight on the upper slide to compress the sample uniformly.

Record the time taken for the upper slide to separate from the lower slide.

$$S=M \times L / T$$

Where, S= Spreadability

M= Weight tide uphill on the slide

L= Length of glass slide

T= Duration of slide separation

Loss on Drying (LOD)[9]

Objective: Determine the amount of moisture lost from the ointment upon drying.

Method

Place a sample of the formulation in a petri dish.

Dry the sample in a water bath under controlled conditions until a constant weight is achieved.

Calculate the LOD as the difference in weight before and after drying, expressed as a percentage.

Solubility[9]

Objective: Assess the solubility characteristics of the ointment.

Method:

Determine solubility in different solvents such as boiling water, alcohol, ether, and chloroform.

Note whether the ointment dissolves, forms a suspension, or remains insoluble in each solvent.

Washability[9]

Objective: Evaluate how easily the ointment can be washed off the skin.

Method:

Apply the ointment to the skin.

Assess the ease and extent of removal by washing with water.

Non-irritancy Test[9]

Objective: Determine if the ointment causes any skin irritation.

Method:

Apply a small amount of the herbal ointment to the skin of human volunteers.

Observe the skin for any signs of irritation, such as redness, itching, or inflammation.

Stability Study[9]

Objective : Assess the physical stability of the ointment under different temperature conditions.

Method :

Store the ointment samples under controlled temperatures (e.g., 2°C, 25°C, 37°C) for four weeks.

Periodically examine the samples for changes in colour, odour,.

Determine if the ointment remains physically stable under these conditions over the study period.

Discussion

The formulation of the herbal ointment appears to meet the required standards based on the evaluation of organoleptic and physiological parameters. This is crucial as it confirms that the ointment is chemically sound and suitable for potential use.

Medicinal Properties:

Historically, herbs such as Neem, Turmeric, Aloe Vera, and lemongrass have been recognized for their medicinal properties, including:

Wound Healing: Accelerating the healing process of wounds.

Antibacterial, Antimicrobial: Fighting against various bacteria and microbes.

Anti-inflammatory: Reducing inflammation, which is beneficial for skin conditions.

Antioxidant: Preventing oxidative damage to skin cells.

Antiseptic, Antifungal: Preventing and treating infections and fungal conditions.

Given these properties, the herbal ointment formulated in this study has the potential to serve as a medium for delivering these medicinal benefits to users. Further studies could explore:

Efficacy Studies: Clinical trials to assess the actual therapeutic effects on wounds, infections, or inflammatory conditions.

Safety Studies: Long-term safety assessments, especially for chronic use.

Comparative Studies: Comparing its efficacy against standard treatments

VIII. SUMMARY [10]

Antiseptic Properties: Many wound healing ointments contain antiseptic agents like iodine, chlorhexidine, or hydrogen peroxide to prevent infection by killing bacteria on the wound surface.

Moisture Retention: Ointments often include ingredients such as petrolatum or lanolin that help keep the wound moist. This moisture can promote faster healing and reduce scarring.

Promotion of Healing: Some ointments contain substances like allantoin, dexpanthenol (provitamin B5), or natural oils (like tea tree oil or calendula) that support the skin's natural healing processes and reduce inflammation.

Protection: Ointments create a barrier over the wound, shielding it from dirt, germs, and further injury, which can facilitate the healing process.

Reduced Scarring: By promoting a moist wound environment and reducing inflammation, certain ointments may help minimize scar formation as the wound heals.

IX. CONCLUSION

Evaluation tests for herbal ointments are research & experiments conducted throughout manufacture that, on occasion, should be conducted post-production by researchers & regulatory bodies. This study investigated and assessed a herbal ointment formulation in terms of physiological characteristics (PH, spreadability, washability, irritancy test, viscosity, loss on drying, centrifugation, solubility) and organoleptic features (appearance, color, and odor). The findings show that the formulation satisfies the norms, proving its chemical soundness. If more research is done, this ointment may prove to have such medicinal qualities. Since neem, turmeric, aloe vera, and lemon grass have been used for centuries for a variety of purposes, including wound healing, anti-bacterial, anti-microbial, anti-inflammatory, anti-oxidant, anti-septic, and anti-fungal properties, so this ointment could become a media for having such medicinal properties if further studies are carried

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