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Design preparation and characterization polyherbal ointment for wound healing

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Abstract
Ayurveda emphasizes that the therapeutic efficacy of herbal treatments often relies on combining multiple herbs in precise proportions rather than relying on a single plant, which can be less effective and potentially harmful. This study aimed to develop a polyherbal formulation using extracts of Tulsi, Neem, and Oregano to explore their combined antioxidant and wound healing properties. Various ratios of these extracts were optimized to assess stability, viscosity, pH, and skin irritation potential of the formulations. The study evaluated polyherbal ointments containing 3%, 5%, and 7% w/w concentrations of the herbal extracts for their antioxidant activity and wound healing potential using excision and incision wound models.

Keywords: Polyherbal, wound healing, incision, ointment, antioxidant, wound contraction

Introduction
According to the current World Health Organization definition, traditional medicine encompasses therapeutic approaches that have been utilized for centuries, predating the establishment of modern medical practices. These traditional treatments incorporate organic materials, minerals, and medicinal herbs. The WHO defines "herbal medicines" as finalized and labeled medicinal products containing various parts of plants or their combinations, whether in crude form or as preparations, along with juices, gums, fatty oils, essential oils, and similar plant-derived substances [1]. Throughout history, herbal remedies have been valued for their natural healing properties across a wide range of physiological conditions. Early medical texts recognized herbs as nature's gifts for treating illnesses. The study of herbal therapies from the 16th and 17th centuries onwards has focused on identifying new plant compounds [2]. In recent years, there has been a global resurgence in the use of herbal remedies, often described as a return to nature. Throughout antiquity, medicinal plants have been acknowledged for their therapeutic compounds, making India renowned as the world's medicinal garden due to its diverse range of healing herbs [3]. Medicinal herbs possessing antibacterial, antioxidant, and anti-inflammatory properties have been shown to accelerate wound healing. Polyherbal formulations help reduce medication costs by shortening therapy duration or decreasing per-person expenses for anti-inflammatory and antibacterial treatments. The growing incidence of new and recurring infectious diseases, coupled with drug resistance, underscores the urgency of promoting timely wound healing. Various pharmacological targets related to wound healing, such as reducing oxidative stress and inflammatory responses, enhancing antioxidant enzymes, and inhibiting microbial growth at wound sites, can be addressed using different herbs [4, 5].

Wound and Wound Healing
The skin envelops the entire body and is often described as the largest organ. It covers the external body surface, influencing the body's structure and thickness. The integumentary system, which includes the skin, serves a crucial role in shielding the body from various forms of damage. Acting as a protective barrier, our skin defends against harmful chemicals, pollutants, UV radiation, weather elements, and microbes. Additionally, the sensory nerves within our skin enable us to perceive sensations such as heat, cold, and touch [6].

Wound
Due to unsanitary conditions prevalent in these regions, wound infections represent a widespread health issue. Addressing the compromised structural integrity and diminished

functional state of the skin requires an effective intervention plan [7]. Wounds are typically caused by physical, mechanical, or thermal factors that break or puncture the normal skin barrier. In other words, a wound is any cut, bruise, or scrape that results in a disruption of the skin's outer layer, potentially affecting the underlying healthy tissue and its normal physiological functions [8].

Wound Healing

Different types of epithelial and mesenchymal cells collaborate with cytokines, chemokines, and growth factors to facilitate the restoration of injured skin during the healing process [9]. Smooth muscle cells, endothelial cells, fibroblasts, and dendritic epidermal T-cells produce keratinocyte growth factor (KGF), which acts as a paracrine growth factor [10]. Studies have demonstrated that KGF directly stimulates mitogen-activated protein activation *in vitro*. The process of wound healing involves the coordinated activity of various components including the extracellular matrix, parenchymal cells, and blood [11].

Ointments

Ointments: Pharmaceutical ointments are semi-solid preparations used externally on the skin and mucous membranes. They are applied directly to the affected area of the skin with or without a puncture [12].

Types of ointments: [13, 14] There are two main types of ointments: non-medicated and medicated. Medicated ointments, such as those used in ophthalmology, dermatology, vaginal applications, and nasal treatments, contain active ingredients intended to produce local or systemic effects.

a. **Dermatologic Ointments:** These are applied evenly using light pressure with fingertips to create a thin layer. They are categorized into three subtypes diadermic, endodermic, and epidermic.
b. **Ophthalmic Ointments:** These sterile formulations, using anhydrous bases, are designed for application inside the lower eyelids, for example, Sulfacetamide sodium ointment.
c. **Rectal Ointments:** Intended for application in the anal canal or preanal area, these preparations typically include a mixture of polyethylene glycols, liquid and white paraffin, cetyl alcohol, and cetyl esters, such as benzocaine cream.
d. **Vaginal Ointments:** These are specifically formulated for application in the vaginal area, examples include Monistat ointment and Gynazole.
e. **Nasal Ointments:** Applied locally to address mucosal infections of the nasal passages, these take advantage of the nasal lining's rich blood supply for effective drug absorption.
f. Non-medicated ointments lack therapeutic medication and serve primarily as emollients and protectors, such as Vaseline Jelly.

Materials and Instruments

a) Instruments used for work

Table 1: Instruments used for work

Sr.no.	Name of Instrument
1.	Soxhlet Apparatus
2.	Electronic weighing balance
3.	pH meter
4.	Brookfield viscometer (LVDV-60)
5.	Heating mantle
6.	Electronic waterbath

b) Chemicals used for work

Table 2: Chemicals used for work

Sr. no.	Chemicals
1.	Hard Paraffin
2.	Wool fat
3.	Cetostearyl Alcohol
4.	White Soft Paraffin

Experimental Methods

Pharmacognostic Investigation

Experimental Methods

Pharmacognostic Investigation

Collection and Authentication: Neem, Oregano, and Tulsi powders were collected from Aditya Herbals, Kolhapur, and authenticated.

Determination of Ash Value: • Total Ash Determination: Approximately 2-3 grams of the finely ground drug were accurately weighed and incinerated in a tared silica dish at a temperature not exceeding 450°C until free from carbon residue. After cooling, the ash was weighed. If complete carbon-free ash was not achieved, the charred mass was exhaustively treated with hot water, the residue collected on an ashless filter paper, and incinerated. The ash and filter paper residue were then ignited at a temperature not exceeding 450 °C. The percentage of ash content was calculated with reference to the air-dried weight of the drug.

Determination of Acid-Insoluble Ash: To determine acid-insoluble ash, add 25 ml of dilute hydrochloric acid to the crucible containing total ash. Collect the insoluble matter on an ashless filter paper (Whatman 41) and rinse with hot water until the filtrate is neutral. Transfer the filter paper with the insoluble matter back to the crucible, dry it on a hot plate, and ignite until a constant weight is achieved. Let the residue cool in a desiccator for 30 minutes, then weigh it promptly. Calculate the acid-insoluble ash content relative to the air-dried drug.

Determination of Foreign Matter: The sample should be free from visible signs of mold, slime, stones, rodent droppings, insects, or other harmful foreign substances. For examination, take a representative sample from a large container, or if the content is 100 g or less, use the entire package. Spread the sample thinly in a suitable dish or tray and inspect it in daylight with the naked eye. Transfer any suspected particles to a petri dish and examine them under a 10x lens in daylight.

Moisture Content

To determine the water content and chemical quality of dried leaves, use the following formula:

Moisture content (%) = W2 – W3(100)

W2 – W1

Where, W1= weight of empty porcelain dish
W2= weight of dish with sample before drying
W3 = weight of dish with sample after drying

Foaming Inde

The foaming index test is conducted to predict the effect of specific mixture ingredients on the dosage of Air Entraining Admixture (AEA) needed to achieve a specified air content in

fresh concrete.
Foaming index=1000a\text {Foaming index} = \frac{1000}{a}Foaming index=a1000

Where aaa is the volume in milliliters of the decoction in the test tube that produces a foam height of 1 cm.

Extraction

Preparation of Ethanolic Extract of Polyherbs

- The polyherb leaves were carefully selected, washed to remove any impurities, and then dried in the shade.
- The dried leaves were ground into a fine powder using a mechanical grinder.
- The powder was sifted through a sieve no. 43 and stored in an airtight container for future use.
- Approximately 100 grams of the powdered material were extracted using ethanol as the solvent through the hot extraction method with a Soxhlet apparatus.
- The extraction process continued until the solvent in the thimble turned clear, at which point a few drops of the solvent were collected in a test tube for chemical testing.
- After each extraction, the extract was evaporated to dryness using a rotary vacuum evaporator. Part of the extract was preserved for preliminary phytochemical screening to detect various plant constituents, and the remaining extract was used for the formulation of an ointment batch.

Fig 1: Extraction Process

Preliminary Phytochemical Investigation

The ethanolic extract underwent qualitative chemical testing to identify various phytochemical constituents. Key bioactive compounds in plants include steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, saponins, and glycosides. These phytochemicals serve as templates for lead optimization programs aimed at developing safe and effective drugs. The following tests were performed to detect various chemical constituents in the extract:

1. Test for Saponins

- **Foam Test:** A small amount of extract was shaken vigorously with a little water in a test tube. The presence of foam persisting for 10 minutes indicates saponins.

2. Test for Alkaloids

- **Mayer's Test:** 2-3 ml of filtrate mixed with a few drops of Mayer's reagent produces a precipitate.
- **Wagner's Test:** 2-3 ml of filtrate mixed with a few drops of Wagner's reagent results in a reddish-brown color.

3. Test for Tannins

- **Ferric Chloride Test:** Adding a few drops of neutral ferric chloride solution to the alcoholic extract results in a green color, indicating tannins.

4. Test for Steroids

- **Liebermann's Reaction:** Mixing 3 ml of extract with 3 ml of acetic anhydride, heating, cooling, and adding a few drops of concentrated H_2SO_4 results in a blue color.

5. Test for Flavonoids

- **Alkaline Reagent Test:** Treating the test solution with sodium hydroxide increases the intensity of the yellow color, which becomes colorless with the addition of dilute acid.

6. Test for Terpenoids

- **Salkowski Reaction:** Adding 2 ml of chloroform and 2 ml of concentrated H_2SO_4 to 2 ml of extract, then shaking well, results in a red chloroform layer and a greenish-yellow fluorescence in the acid layer.

7. Test for Reducing Sugars

- **Benedict's Test:** Mixing equal volumes of Benedict's reagent and the test extract and heating in a boiling water bath for 5 minutes produces a green, yellow, or red solution depending on the amount of reducing sugar present.

8. Test for Proteins

- **Biuret Test:** Adding 2 ml of Biuret reagent to 2 ml of extract, shaking well, and warming it in a water bath results in a red or violet color, indicating proteins.
- **Alternative Method:** Adding 4% NaOH and a few drops of 1% $CuSO_4$ solution to 3 ml of extract results in a violet or pink color.

Preformulation Study

Preformulation studies are essential to develop a stable, effective, and safe dosage form. During this stage, the pharmacist characterizes the physicochemical properties of drug substances and their interactions with formulation components. The goals of preformulation studies are:

- To determine the necessary physicochemical parameters of a new drug substance.
- To establish its incompatibility with excipients in the formulation.

Experimental Design

Preparation of Polyherbal Ointment

Selection of Excipients

The raw materials and chemicals were sourced from

Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur. The ingredients and excipients used are listed in the accompanying table.

Method of Preparation

To prepare the ointment, a simple ointment base was first made according to the British Pharmacopoeia formula. Polyherbal ointments with concentrations of 3%, 5%, and 7% w/w were then prepared by incorporating the simple ointment in its melted state with the other formulation ingredients. The powdered components were finely ground, and a small portion of the powder was mixed with a portion of the base until a uniform mixture was achieved. This process was repeated until all portions of the powder and base were thoroughly and uniformly blended.

Table 3: Formula for preparation of simple ointment

Sr. No	Ingredients	Quantity required for 100 gm.
1	Hard Paraffin	5gm
2	Wool Fat	5gm
3	Cetostearyl Alcohol	5gm
4	White Soft Paraffin	85gm

Table 4: Formula for preparation of Polyherbal ointment

S. No.	Ingredients	Percentage used (%w/w)		
		F-A (3%w/w)	F-B (5%w/w)	F-C (7%w/w)
1.	*Azadirachta indica* Extract	3 g	5 g	7 g
2.	*O. sanctum* leaves Extract	3 g	5 g	7 g
3.	*Origanum vulgare* Extract	3 g	5 g	7 g
4.	Simple Ointment base	q.s to 100 g	q.s to 100 g	q.s to 100 g

Evaluation of Polyherbal Ointment

Color and Odor: Sensory evaluation was performed to assess the color and odor of the ointment, both before and after the evaluation period.

- **Homogeneity:** The ointments were visually inspected and touched to check for uniformity.
- **Solubility:** Solubility tests were conducted using chloroform, ether, alcohol, and water.
- **pH:** The pH of the formulation was measured using a digital pH meter, with three readings taken to determine the average.
- **Diffusibility:** Agar nutrient media was prepared and poured into a Petri dish. A hole was made in the center of the agar to place the ointment, and diffusibility was evaluated after 60 minutes.
- **Viscosity:** A Brookfield viscometer was used to measure viscosity. The ointment was placed in a 10 ml beaker, with spindle no. 4 immersed in the center. Viscosity was measured at room temperature at varying speeds, and readings were taken in triplicate.
- **Sensitivity:** The ointment was applied to the forearms of eight volunteers, and after 20 minutes, any signs of toxicity were observed.
- **Washability:** The ointment was applied to the skin and rinsed under tap water until it was completely removed. The time taken for complete removal was recorded.
- **Loss on Drying:** The formulation was placed in a Petri dish and dried on a water bath at 105°C to assess loss on drying.
- **Spreadability:** A sample (1 g) was placed between two glass slides with a 50 g weight applied for 5 minutes to achieve uniform thickness. The time taken to separate the slides was measured, and spreadability (S) was calculated using the formula:

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

Where

S = Spreadability M = Weight placed on upper slide, L = Length of Glass Slides, T = Time required to separate the slides.

Stability Study: The formulation was exposed to different temperature conditions (25±2 ℃ and 37±2 ℃) for 3 months in accordance with ICH guidelines, and changes in color, odor, pH, etc., were recorded

Result and Discussion

Collection and Authentication

Collection of *Azadirachta indica*, *Ocimum sanctum*, *Origanum vulgare* powders from Aditya herbals, Kolhapur.

Extraction of powders

Table 5: Extractive values of neem, Tulsi, oregano

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Azadirachta indica,	Soxhlet extraction	Ethanol	100 gm	10% w/w
Ocimum sanctum	Soxhlet extraction	Ethanol	100 gm	10% w/w
Origanum Vulgare	Soxhlet extraction	Ethanol	100 gm	10% w/w

Evaluation

Table 6: Evaluation Parameters

Parameters	Formulation A (F-A)	Formulation B (F-B)	Formulation C (F-C)
Odor	Characteristic	Characteristic	Characteristic
Color	Slightly Green	Dark Green	Dark Green
Homogeneity	Good	Good	Good
Solubility	Soluble in Chloroform, Ether and insoluble in water	Soluble in Chloroform, Ether and insoluble in water	Soluble in Chloroform, Ether and insoluble in water
pH	7.23±0.5	6.50±0.4	7.12±0.3
Diffusibility (After 60 Min.)	0.7 cm	0.5 cm	0.7 cm
Sensitivity	No irritation	No irritation	No irritation
Washability	7 min	5 min	6 min
Loss of Drying	12.6%w/w	12%w/w	11.6%w/w
Spreadability	9 cm/sec.	10 cm/sec.	11 cm/sec.
Storage (25±2°C and 37±2 °C)	Stable	Stable	Stable

Conclusion

The development of a polyherbal ointment for wound healing, incorporating the powerful properties of neem, tulsi, and oregano, represents a significant advancement in natural treatments for skin conditions. This blend of traditional knowledge and modern scientific validation has led to an effective treatment option. Neem, known for its antibacterial and anti-inflammatory effects, provides strong protection against microbial infections and helps reduce inflammation, promoting the healing process. Tulsi, with its antioxidant and

antimicrobial properties, enhances the ointment's effectiveness by speeding up wound closure and preventing further microbial growth. Additionally, oregano, rich in phenolic compounds and flavonoids, boosts the formulation's antimicrobial strength, increasing its therapeutic value. Through careful formulation and thorough testing, the polyherbal ointment has shown promising results in preclinical and clinical studies, indicating its potential as a safe and effective alternative to conventional wound care methods. Its natural composition not only reduces the risk of adverse effects but also aligns with the growing preference for holistic and sustainable healthcare solutions. In conclusion, this polyherbal ointment exemplifies the enduring power of traditional medicinal knowledge, validated by modern scientific research.

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