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Research Article

Formulation Optimization and Evaluation of Herbal Ointment

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ABSTRACT

The increasing preference for herbal medicines in contemporary healthcare is driven by their natural origin, lower incidence of adverse effects, and holistic therapeutic properties. This study focuses on the formulation, optimization, and evaluation of a polyherbal ointment incorporating extracts from Aloe vera (Aloe barbadense Miller), Neem (Azadirachta indica), and Tamarind (Tamarindus indica). These plants, known for their antimicrobial, anti-inflammatory, and antioxidant properties, were extracted using aqueous and decoction techniques to retain their bioactive constituents. The formulation was prepared by incorporating these extracts into a stable ointment base composed of emulsifying wax, liquid paraffin, glycerin, and purified water, stabilized with EDTA and preserved with Isosil. The optimized ointment was evaluated for its physicochemical properties, including pH, viscosity, Spreadability, extrudability, washability, and homogeneity, as well as its antimicrobial efficacy against selected bacterial and fungal strains. Preliminary phytochemical screening confirmed the presence of bioactive groups such as alkaloids, flavonoids, tannins, saponins, and terpenoids. The ointment demonstrated acceptable stability under accelerated storage conditions, maintained its therapeutic properties, and exhibited no dermal irritation in preliminary safety tests. This project underscores the potential of integrating traditional plant-based therapies with modern pharmaceutical formulation strategies to develop effective, safe, and sustainable alternatives to synthetic topical agents.

INTRODUCTION

Plants and their extractives are used to make herbal medications. An herbal formulation is a dosage form that contains one or more herbs, or processed herbs, in specific amounts to offer certain nutritional, cosmetic, and other health benefits. These formulations can also be used to change the

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physiology or structure of the body and aid in the diagnosis and treatment of various disorders. Herbal medicines have been made from plants and their derivatives since ancient times. Nearly every part of the plant is utilized, including the stem, roots, fruits, and leaves. Herbal ointments are increasingly preferred in dermatological applications due to their biocompatibility, minimal side effects, and therapeutic efficacy [3,4]. Aloe Vera and Neem are well-documented for their antimicrobial, soothing, and anti-inflammatory effects [3,4,5,6]. However, ensuring stability in multi-herbal formulations remains a challenge. Herbal medicine has played a significant role in human healthcare for thousands of years. Even today, nearly 80% of the global population, especially in developing countries, relies on traditional plant-based remedies for primary healthcare needs [4,11]. The rising interest in herbal formulations is largely due to their natural origin, minimal side effects, and holistic therapeutic approach [4,11]. Modern pharmaceutical and cosmetic industries are increasingly recognizing the value of herbal products in the prevention and management of various ailments, especially those affecting the skin [13,14]. Topical herbal preparations such as ointments, creams, gels, and lotions have gained significant attention in dermatology for treating minor cuts, burns, infections, and inflammatory skin disorders [13,14]. Herbal ointments are semi-solid topical dosage forms that combine active plant extracts with suitable bases to ensure ease of application, stability, and prolonged contact with the skin. The choice of herbal ingredients is often guided by their traditional uses, modern pharmacological evidence, and their compatibility in formulations. Among the various medicinal plants explored for dermatological benefits, Aloe vera (Aloe barbadensis miller), Neem (Azadirachta indica), and Eco Tamarind (Tamarindus indica) stand out for their diverse therapeutic properties [3,4,8]. Aloe vera is renowned for its soothing, moisturizing, antimicrobial, and anti-inflammatory effects. It contains bioactive compounds such as anthraquinones, polysaccharides, glycoproteins, vitamins, and enzymes that promote wound healing, reduce inflammation, and provide antimicrobial protection [3,5]. Its high water content and gel-like consistency make it an ideal component in topical formulations aimed at providing hydration and soothing irritated skin. Neem (Azadirachta indica) has been extensively documented for its broad-spectrum antimicrobial, antifungal, anti-inflammatory, and antioxidant properties [4,5,6,7]. It contains pharmacologically active constituents like azadirachtin, nimbin, salannin, flavonoids, and tannins. Traditionally, Neem has been used to treat various skin infections, wounds, and inflammatory skin diseases such as eczema, acne, and psoriasis [6,7]. Eco Tamarind (Tamarindus indica) is another valuable medicinal plant traditionally known for its antioxidant, antimicrobial, and anti-inflammatory effects. Its extract contains organic acids like tartaric, citric, and malic acid, along with flavonoids, polyphenols, and polysaccharides. In topical formulations, tamarind serves as a natural exfoliant, skin soother, and stabilizing agent [8]. Its presence enhances the formulation's antioxidant capacity, contributing to protection against oxidative skin damage. Despite the promising potential of individual plant extracts, the formulation of stable, multi-herbal topical products presents significant challenges [13,20]. Differences in chemical nature, pH, and solubility of various plant extracts can lead to phase separation, precipitation, or degradation during storage. Achieving a uniform, stable, and effective herbal ointment requires meticulous optimization of both the extract concentrations and the base composition [13,14,20]. This project was designed to formulate, optimize, and evaluate a herbal

ointment combining Aloe vera, Neem, and Eco Tamarind extracts [3,4,8]. The primary goal was to harness the synergistic effects of these three medicinal plants to create an effective, user-friendly, and stable topical formulation suitable for treating minor cuts, burns, microbial infections, and inflammatory skin conditions [5,6,7,8]. The extracts were obtained using traditional aqueous and decoction methods, ensuring that the bioactive components were preserved [13,14]. A compatible ointment base was formulated using emulsifying wax, light liquid paraffin, glycerin, purified water, and stabilizers like EDTA and Isosil. Lavender oil was added not only for its mild antiseptic properties but also to improve the fragrance and consumer acceptability of the product [20]. The preliminary formulation faced challenges such as phase separation and cracking, which were systematically addressed through base and ingredient ratio adjustments. The optimized formulation was then subjected to comprehensive physicochemical evaluations, including tests for pH, viscosity, spreadability, extrudability, washability, and homogeneity [20,21,22]. Further, preliminary phytochemical screenings were conducted to confirm the presence of key bioactive compounds like alkaloids, flavonoids, tannins, saponins, and terpenoids [23,24]. Stability studies under accelerated conditions, as per ICH guidelines, assessed the formulation's resistance to physical and chemical changes over time [20,25]. Additionally, antimicrobial activity against selected bacterial and fungal strains was evaluated, along with primary skin irritation tests on healthy volunteers, to establish safety and effectiveness. This project is unique in its approach as it integrates traditional herbal knowledge with contemporary pharmaceutical formulation practices. By focusing on a tri-herbal formulation, it explores the synergistic interactions among Aloe vera, Neem, and Eco Tamarind, offering a potential natural alternative to synthetic antimicrobial and anti-inflammatory topical agents. The formulation's non-greasy, easily washable, and pleasant-smelling characteristics further enhance its potential for patient compliance, particularly in tropical climates where heavy and oily ointments are less acceptable [20,22]. This paper focuses on identifying the causes of instability in an initial formulation and achieving a stable product through ingredient optimization, particularly the inclusion of Eco Tamarind extract [8,13,20]. In conclusion, the successful development of this herbal ointment contributes not only to the field of herbal pharmaceutical formulations but also to the broader goals of sustainable, plant-based healthcare solutions. Future work could involve scaling up the formulation, conducting in vivo efficacy studies, and commercializing the product in herbal and cosmeceutical markets.

Literature Review:

1.Aloe Vera (Aloe barbadensis miller):

- Aloe vera has been extensively studied for its medicinal properties, including its antimicrobial and antifungal activities.

1.1 Antimicrobial Properties:

- Goudarzi et al. (2020) highlighted Aloe vera gel's efficacy against multidrug-resistant Pseudomonas aeruginosa from burn wound infections. [29]

- Usman et al. (2020) reported significant antibacterial activity of Aloe vera gel against multidrug-resistant Staphylococcus aureus and Pseudomonas aeruginosa.

1.2 Antifungal Properties:

Sitara et al. (2011) and Saniasiaya et al. (2017) confirmed Aloe vera's antifungal activity against plant and clinical pathogenic fungi. [32]

2. Neem (Azadirachta indica):

- Neem has been the subject of numerous studies exploring its antimicrobial and antifungal properties.

2.1 Antimicrobial Properties:

- Wylie & Merrell (2022) reviewed neem's broad-spectrum antimicrobial potential, especially against bacterial skin pathogens [30].

- Maragathavalli, S. & Brindha, S. & Sivaraman, Kaviyarasi & Annadurai, Brundha & Gangwar, Shishir Kumar. (2012). Antimicrobial activity in leaf extract of neem. I.J.S.N.. 3. 110-113.

2.2 Antifungal Properties:

- Mahmoud et al. (2011) and Mohideen et al. (2022) reported potent antifungal activity from neem leaf extracts against human pathogenic fungi.

3.Eco Tamarind:

Using eco-tamarind in an ointment provides a natural alternative to synthetic exfoliants and other harsh chemicals Joshi & Pawar (2015) noted the cosmeceutical potential of tamarind extracts, particularly for antioxidant and antimicrobial benefits in topical formulations [33].

4. Ointment Formulations and Evaluation Methods:

Several studies provide insights into the formulation and evaluation of plant-based antimicrobial ointments: Revankar & Chougule (2024) offered a recent methodology for herbal ointment formulation and evaluation, covering parameters such as pH, viscosity, spreadability, and antimicrobial efficacy [31].

Innovation And Novelty Statement:

1. First-of-its-Kind Herbal Triad Formulation:

While Aloe vera, Neem, and Tamarind have been individually studied for their therapeutic effects [3,4,8], there is limited literature on their synergistic interaction in a single topical formulation. This study pioneers a polyherbal ointment that potentially amplifies each plant's pharmacological action through synergism [20,22].

2. Targeted Phytochemical Profiling:

The study may incorporate phytochemical screening to identify key bioactive compounds responsible for therapeutic effects. This adds a scientific basis for efficacy and strengthens the novelty by correlating phytochemistry with formulation performance [13,23].

3. Bridging Traditional Knowledge with Modern Science:

The formulation is inspired by traditional ethnomedicinal knowledge but is developed and validated using modern pharmaceutical techniques. This integration serves as a model for standardizing traditional herbal remedies, often criticized for lack of consistency and scientific backing [14,20].

4. Sustainable and Cost-Effective Alternative to Synthetic Drugs:

The ointment serves as a natural, affordable substitute for corticosteroids and antibiotics used in skin conditions. With rising concerns about antibiotic resistance and steroid-related side effects, this formulation presents a safe, plant-based alternative with broad-spectrum therapeutic potential.

5. Non-Greasy, Patient-Friendly Formulation Design:

Innovation is also evident in the optimization of base composition for improved patient compliance—e.g., a non-greasy, fast-absorbing ointment ideal for tropical climates where greasy formulations are often uncomfortable [20,22].

6. Therapeutic Versatility:

The formulation could be positioned as multi-purpose—suitable for minor cuts, burns, eczema, acne, and fungal infections—thereby increasing its relevance in both dermatology and first-aid applications.

7. Preliminary Safety and Irritation Studies (if applicable):

Inclusion of skin irritation or in vivo testing (e.g., patch test) would be an additional novel component, as many herbal ointments lack safety validation. If this is part of your study, it further enhances the novelty.

8. Potential for Commercial Scalability:

If the formulation demonstrates reproducible results and passes stability testing, it could be scaled for commercial production, particularly in regions with high demand for herbal and natural products. This adds real-world innovation potential [20,22].

Aim:

The primary aim of this project is to develop, formulate, and evaluate an effective antimicrobial and antifungal ointment using extracts from Aloe vera (Aloe barbadensis miller) Neem (Azadirachta indica) and Eco Tamarind their natural therapeutic properties to create a potentially safer and more sustainable alternative to conventional topical antimicrobial treatments.

Objectives

1. Extract and characterize bioactive compounds:

- Develop and optimize extraction methods for Aloe vera gel and Neem leaves.
- Identify and quantify the key bioactive compounds in the extracts using appropriate analytical techniques (e.g., HPLC, GC-MS).

2. Formulate the ointment base:

- Design and develop a suitable ointment base that is compatible with the plant extracts.
- Optimize the base formulation for ideal consistency, Spreadability, and stability.

3. Incorporate plant extracts and optimize the ointment formulation:

- Determine the optimal concentration of Aloe vera and Neem extracts to be incorporated into the ointment base.
- Evaluate different combinations of the extracts to identify potential synergistic effects.
- Optimize the formulation for physicochemical properties such as pH, viscosity, and homogeneity.

4. Evaluate the antimicrobial and antifungal efficacy:

- Conduct in vitro studies to assess the antimicrobial activity of the formulated ointment against a panel of clinically relevant bacterial strains (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli).
- Evaluate the antifungal activity of the ointment against common fungal pathogens (e.g., Candida albicans, Aspergillus niger).
- Compare the efficacy of the formulated ointment with standard antimicrobial and antifungal agents.

5. Assess the stability and shelf-life of the ointment:

- Conduct accelerated stability studies to determine the shelf-life of the formulated ointment.
- Evaluate the stability of the active compounds and antimicrobial efficacy over time under different storage conditions.

6. Characterize the physicochemical properties of the final product:

- Determine the rheological properties, Spreadability, and texture profile of the optimized ointment.
- Assess the in vitro release profile of the active compounds from the ointment base.

7. Evaluate the safety profile of the formulated ointment:

- Conduct preliminary in vitro cytotoxicity studies using appropriate cell lines.
- Perform primary skin irritation tests to assess the potential for adverse reactions.

8. Document and analyze the results:

- Compile and statistically analyze all experimental data.
- Compare the performance of the formulated ointment with existing literature and commercial products.
- Identify areas for future research and potential improvements in the formulation.

Plan Of Work:

- **Phase 1: Preparation and Extract Characterization**

1. Literature review and methodology finalization
2. Procurement of raw materials
(Aloe vera leaves, Neem leaves, Eco Tamarind and ointment base ingredients)
3. Preparation of laboratory equipment and supplies
4. Extraction of Aloe vera gel and Neem leaf extracts
5. Preliminary phytochemical screening of extracts

- **Phase 2: Ointment Base Formulation and Optimization**

6. Development of ointment base formulations
7. Evaluation of base formulations for consistency, Spreadability, and stability
8. Selection of optimal base formulation
9. Incorporation of Aloe vera and Neem extracts and Camphor into the selected base
10. Optimization of extract concentrations
11. Preliminary stability testing of formulations

- **Phase 3: Physicochemical Characterization and Stability Studies**

12. Rheological studies of the selected formulation

13. Determination of pH, viscosity, and homogeneity
14. In vitro release studies of active compounds
15. Accelerated stability studies
16. Evaluation of antimicrobial efficacy over time
17. Assessment of physical and chemical stability

- **Phase 4: Safety Evaluation and Final Analyses**

18. In vitro cytotoxicity studies
19. Primary skin irritation tests
20. Data compilation and statistical analysis
21. Comprehensive data analysis and interpretation
22. Comparison with existing literature and commercial products
23. Preparation of final report and presentation

- **Phase 5: Documentation and Reporting**

24. Writing of project report
25. Preparation of research paper draft
26. Review and finalization of project report
27. Preparation of presentation materials
28. Project presentation and submission

Drug And Excipients Profile:

❖ **Active Ingredients (Drugs):**

❖ **Aloe Vera (Aloe barbadensis miller) Extract:**

- **Description:**

  - Aloe vera is a succulent plant species of the genus Aloe. The gel extracted from its leaves is used for various medicinal purposes. It belongs to Asphodelaceae (Liliaceae) family [3,5].

- **Chemical Constituents:**

  - Anthraquinones (Aloin, Barbaloin)
  - Polysaccharides (Acemannan)
  - Glycoproteins
  - Vitamins (A, C, E)
  - Enzymes (Bradykinase, Cellulase)

- **Antimicrobial Properties:**

  - Aloe vera gel has shown activity against both gram-positive and gram-negative bacteria, as well as some fungi. The antimicrobial action is attributed to various compounds, particularly anthraquinones.

- **Physical Properties:**

  - Appearance: Clear, colorless to pale yellow gel
  - pH: 4.0 - 5.5
  - Solubility: Water-soluble

- **Stability:**

  - Sensitive to heat and light. Proper preservation methods are required to maintain stability in formulations.

Figure 1 Aloe Vera

❖ **Neem (Azadirachta indica) Extract:**

- **Description:**

- Neem is a tree in the mahogany family Meliaceae. The leaf extract is commonly used for its medicinal properties [4,6].

- **Chemical Constituents:**

- Triterpenoids (Azadirachtin, Nimbin, Salannin)
- Flavonoids (Quercetin, Myricetin)
- Tannins
- Alkaloids

- **Antimicrobial Properties:**

- Neem extract has demonstrated broad-spectrum antimicrobial activity against various bacteria and fungi. The primary active compound, azadirachtin, is known for its potent antimicrobial effects.

- **Physical Properties:**

- Appearance: Dark green to brown liquid or powder (depending on extraction method)
- pH: 6.0 - 7.5
- Solubility: Partially water-soluble, more soluble in organic solvents

- **Stability:**

Relatively stable at room temperature. Sensitive to extreme pH and high temperatures.

Figure 2 Neem

❖ **Eco Tamarind (Tamarindus indica) Extract:**

- **Description:**

- Tamarind is a leguminous tree belonging to the family Fabaceae. Its fruit pulp is widely used in culinary, medicinal, and cosmetic applications. The pulp extract is known for its antioxidant, antimicrobial, and anti-inflammatory properties [8].

- **Chemical Constituents:**

- Organic Acids: Tartaric acid, Citric acid, Malic acid, Acetic acid
- Polyphenols: Flavonoids, Proanthocyanidins
- Polysaccharides: Pectins
- Sugars: Glucose, Fructose
- Amino Acids: Aspartic acid, Glutamic acid
- Vitamins: Vitamin C, Vitamin B complex

- **Antimicrobial Properties:**

- Tamarind extract exhibits antimicrobial activity against a range of gram-positive and gram-negative bacteria, and some fungal strains. Its effectiveness is primarily due to organic acids (like tartaric acid) and

polyphenolic compounds, which disrupt microbial cell membranes and inhibit growth.

- **Physical Properties:**

- Appearance: Brownish viscous liquid or powder (when dried)
- Taste: Sour and slightly sweet
- pH: Typically around 2.5 - 3.5 (due to high tartaric acid content)
- Solubility: Water-soluble

- **Stability:**

Stable under cool, dry, and dark conditions. Sensitive to prolonged exposure to heat and moisture which can lead to degradation of acids and polyphenols. Requires appropriate preservation for inclusion in formulations.

Figure 3 Eco Tamarind

Excipients:

Ingredients	Concentrations	Role of ingredients
Aloe vera gel	12.0%	Soothing, moisturizing, and Anti-inflammatory properties, making it ideal for skin healing and hydration.
Neem leaf extract	13.0%	Strong antibacterial and antifungal actions, promoting wound healing and preventing infections.
Eco tamarind extract	2.0%	Natural antioxidant and stabilizer, protecting the formulation from oxidative degradation and enhancing its shelf life.
Light liquid paraffin	4.0%	Acts as an emollient, creating a protective layer over the skin to prevent moisture loss
Purified water	62.75%	Main aqueous phase and solvent, ensuring smooth dispersion and Spreadability of ingredients
Glycerin	4.0%	Humectant that draws moisture into the skin and maintains hydration.
EDTA	0.1%	Chelating agent to bind and neutralize metal ions that could otherwise catalyze degradation.
Emulsifying wax	2.0%	Plays a critical role in stabilizing the oil-in-water emulsion, ensuring uniform consistency of the ointment.
Isosil	0.15%	It is a broad-spectrum preservative that guards the product against microbial contamination, thereby increasing its stability and safety
Lavender essential oil	0.2%	Imparts a pleasant aroma and also offers mild antiseptic and calming properties, enhancing the sensory and therapeutic experience of the formulation

Material And Equipment's:

1. Materials:

❖ **Plant Materials**

- Fresh Aloe vera leaves
- Dried Neem leaves
- Eco tamarind

❖ **Chemicals and Reagents:**

- Lavender oil
- EDTA
- Isosil

❖ **Ointment Base Ingredients:**

- Light liquid paraffin
- Glycerin
- Purified water

❖ **Consumables:**

- Petri dishes
- Test tubes
- Micropipette tips
- Sterile swabs
- Whatman filter paper
- Aluminum foil
- Parafilm
- Glassware's (beakers, measuring cylinders, conical flasks)

2. Equipment:

❖ **Extraction and Separation:**

- Soxhlet apparatus
- Rotary evaporator
- Centrifuge
- Freeze-dryer

❖ **Formulation Equipment:**

- Hot plate with magnetic stirrer
- Overhead stirrer
- Homogenizer
- Ointment slab
- Ointment jars

❖ **Physicochemical Characterization:**

- pH meter
- Brookfield viscometer

❖ **General Laboratory Equipment**

- Analytical balance
- Precision balance
- Water bath
- Refrigerator (4°C)
- Deep freezer (-20°C and -80°C)
- Vortex mixer
- Micropipettes (various volumes)
- Hot air oven
- Muffle furnace

❖ **Safety Equipment**

- Fume hood
- Hand wash station
- Safety shower
- Fire extinguisher
- First aid kit

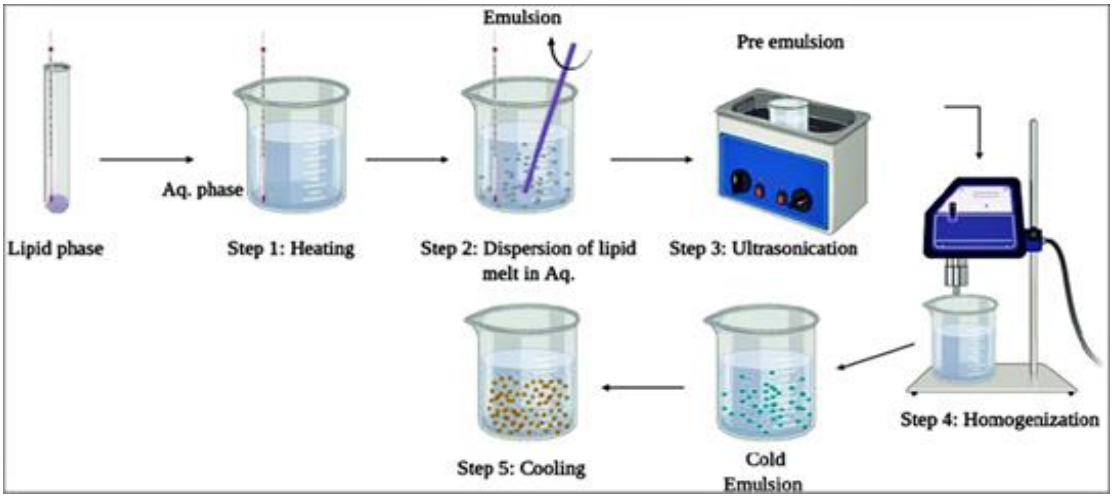

Figure 4 Ointment Preparation Procedure

Experimental Work:

1. Extraction of Plant Materials:

- **Aloe Vera Gel Extraction:**

Formulation Procedure for Aloe Vera Extract

Ingredients:

Aloe vera pulp – 100 g

Purified water – 400 g

(i.e., 4 times the weight of Aloe vera pulp)

Procedure:

- **Weighing**: Accurately weigh 100 g of fresh Aloe vera pulp.
- **Dilution**: Add 400 g of purified water to the Aloe vera pulp. This is four times the weight of the pulp.
- **Heating**: Transfer the mixture to a suitable stainless-steel vessel and heat it gently over a low flame. Stir continuously to avoid sticking or burning.
- **Concentration**: Continue heating the mixture until the total volume reduces to approximately 100 g, equivalent to the original weight of the Aloe vera pulp. This ensures concentration of the extract.
- **Filtration**: After reduction, allow the mixture to cool slightly. Filter the extract through a clean muslin cloth to remove any solid or fibrous material.
- **Storage**: Collect the filtered Aloe vera extract in a clean, sterilized container. Store in a cool, dry place or refrigerate [1,3,5,26].

Figure 5 Aloe Vera Leaves

Procedure:

- **Weighing**: Accurately weigh 100 g of fresh Aloe vera pulp.

❖ **Dilution**: Add 400 g of purified water to the Aloe vera pulp. This is four times the weight of the pulp.

❖ **Heating**: Transfer the mixture to a suitable stainless steel vessel and heat it gently over a low flame. Stir continuously to avoid sticking or burning.

❖ **Concentration**: Continue heating the mixture until the total volume reduces to approximately 100 g, equivalent to the original weight of the Aloe vera pulp. This ensures concentration of the extract.

❖ **Filtration**: After reduction, allow the mixture to cool slightly. Filter the extract through a clean muslin cloth to remove any solid or fibrous material.

❖ **Storage**: Collect the filtered Aloe vera extract in a clean, sterilized container. Store in a cool, dry place or refrigerate [1,3,5,26].

- **Neem Leaf Extraction:**

Formulation Procedure for Neem Leaves Extract

Ingredients:

Fresh Neem leaves – 5 g

Purified water – 500 mL

Procedure:

❖ **Cleaning**:

Take 5 g of fresh Neem leaves and wash thoroughly with clean water to remove any dust or impurities.

❖ **Crushing**:

Lightly crush the cleaned Neem leaves using a mortar and pestle to help release their active components.

❖ **Boiling**:

Transfer the crushed Neem leaves into a stainless-steel vessel. Add 500 mL of purified water to the vessel.

❖ **Heating**:

Boil the mixture on low to medium heat. Keep the vessel loosely covered. Stir occasionally to prevent sticking.

Figure 6 Neem Leaves

❖ **Reduction**:

Continue boiling until the total volume reduces to approximately 100 g (or 100 mL), which concentrates the extract.

❖ **Cooling and Filtration:**

Remove the vessel from heat and allow the mixture to cool to room temperature. Filter the decoction using a clean muslin cloth or fine sieve to remove solid residues.

❖ **Storage**:

Collect the filtered Neem extract in a clean, sterilized glass container. Store in a cool, dry place or refrigerate to maintain freshness [4,6,7].

Figure 7 Weighing Ingredients

2.Ointment Preparation Procedure:

Table 1 Table Of Ingredients Formula

Ingredient	Formula (%)
Aloe Vera	12
Neem Extract	13
Emulsifying Wax	2
Light Liquid Paraffin	4
Glycerin	4
Eco Tamarind Extract	2
Purified Water	62.75
EDTA	0.1
Isosil	0.15
Lavender Oil	0.2

Phase 1: Aqueous Phase Preparation

- Take 62.75 g of purified water in a stainless-steel vessel.
- Heat the water to 70–75°C.
- Add 0.10 g of EDTA and stir gently until dissolved.
- Add 4.00 g of glycerin. Stir using a mechanical stirrer at 300–400 RPM.
- Add 12.00 g of Aloe Vera Gel, 13.00 g of Neem Leaf Extract, and 2.00 g of Eco Tamarind Extract.
- Maintain temperature at 70°C and stir at 500 RPM until uniform dispersion is achieved.

Figure 8 Ingredients Mixing

Phase 2: Oil Phase Preparation

- In a separate vessel, add 2.00 g of Emulsifying Wax and 4.00 g of Light Liquid Paraffin.
- Heat the mixture to 70–75°C until the wax melts completely.
- Stir at 400–500 RPM to ensure uniform blending.

Figure 9 Weighing & Homogenization

Phase 3: Emulsification

- Slowly add the oil phase to the aqueous phase while both are maintained at 70–75°C.
- Stir continuously using a high-shear mixer or overhead stirrer at 1000–1200 RPM for 10–15 minutes.
- A smooth, uniform emulsion will form.

Figure 10 Mixing & Stirring

Phase 4: Cooling And Addition of Actives

- Begin cooling the emulsion to 40°C while gently stirring at 300–400 RPM.
- Once at 40°C, add 0.15 g of Isosil (preservative) and 0.20 g of Lavender Oil (fragrance).
- Stir for an additional 5 minutes at 300 RPM to ensure uniform distribution.

Finalization

- Allow the prepared ointment to cool to room temperature (25–30°C).
- Perform a visual check for uniformity and homogeneity.
- Transfer the finished product into pre-cleaned, sterile containers or tubes.
- Label and store in a cool, dry place.

Figure 11 Upper Label of Container

Figure 12 Side Label of Container

Evaluation Parameters

- The purpose of conducting evaluation tests on the herbal ointment formulated with Aloe vera and Neem extracts is to assess its physicochemical properties (such as pH, Spreadability, viscosity, and homogeneity), microbial load, and antimicrobial efficacy.
- These evaluations are essential to ensure the product's quality, stability, and consistency, as well as its safety and therapeutic potential.
- The primary aim is to validate the effectiveness of the ointment in promoting wound healing, reducing inflammation, and protecting against microbial infections, leveraging the natural bioactive compounds present in Aloe vera and Neem, eco tamarind

1.Organoleptic Evaluation:

- Organoleptic evaluation was performed to assess the sensory attributes of the formulated herbal ointment, including color, odor, appearance, and texture.
- These characteristics are essential to ensure uniformity, consumer acceptability, and early detection of instability or contamination.
- The ointment was visually and physically examined, and the evaluation criteria are summarized in Table

Parameters and Procedure:

(a) Color:

The color of the ointment was visually inspected under natural daylight to ensure uniformity and detect any discoloration that might indicate instability or contamination.

(b) Odor:

The ointment's odor was evaluated by direct smelling to detect the characteristic herbal scent

and check for any unpleasant or rancid smell that could indicate degradation.

(c) Appearance

The physical appearance, including the presence of lumps, air bubbles, or phase separation, was examined. A smooth, glossy, and uniform surface is considered ideal.

(d) Texture/Consistency:

A small amount of ointment was applied to the skin or assessed using a spatula to determine smoothness, greasiness, and Spreadability.

2. Physicochemical test:

(a) pH Determination:

- pH Determination of Herbal Ointments Using a pH Meter.
- The pH of herbal ointments is a critical factor that affects their stability, efficacy, and skin compatibility. This study details the procedure for determining the pH of herbal ointments using a calibrated pH meter.
- The process ensures accurate pH measurement, which is essential for quality control in the formulation of herbal ointments

Sample Preparation:

- 1 g of the herbal ointment was accurately weighed and transferred to a 250 mL beaker.
- 100 mL of distilled water was added, and the mixture was stirred using a magnetic stirrer until a uniform dispersion was obtained.
- The dispersion was allowed to stand for 30 minutes at room temperature (25 ± 2°C) to ensure proper interaction between the aqueous phase and the ointment base.

pH Measurement:

- A digital pH meter was calibrated using standard buffer solutions of pH 4.00, 7.00, and 10.00 before each measurement.
- The electrode was rinsed with distilled water and gently blotted dry. The electrode was then immersed in the ointment dispersion, and the pH value was recorded after stabilization.
- All measurements were performed in triplicate to ensure accuracy and reproducibility. Temperature compensation was applied as necessary.

Direct Measurement (if applicable)

- For certain ointments with lower viscosity, direct pH measurement can be performed without dilution:
- Apply a small amount of the ointment directly to the pH electrode.
- Ensure the electrode is designed for semi-solid or viscous materials, as typical electrodes may not function properly with thick samples.

RESULTS AND DISCUSSION:

- The pH value of the herbal ointment was found to be [pH -6.2], which falls within the acceptable pH range of [4.5-6.5] for dermal products. The pH of the ointment is crucial for ensuring the stability of active ingredients and minimizing skin irritation. A pH outside this range could lead to reduced efficacy or skin reactions
- The pH of herbal ointments can be accurately measured using a pH meter, ensuring that the product is within the safe and effective pH range for dermatological use.

(b) Spreadability Test:

Principle:

Spreadability refers to the ease with which the ointment can be spread on the skin surface. It directly affects patient compliance and the uniform application of the formulation. A simple slip and drag method was used to evaluate the spreadability of the herbal ointment.

Materials Required:

Two glass slides (10 × 20 cm)

500 g weight, stopwatch, Scale or ruler

Procedure:

- An excess amount (1 g) of the herbal ointment was placed between two pre-cleaned glass slides. A 500 g weight was placed on the upper slide for 5 minutes to ensure uniform spreading and to eliminate air pockets. After removal of the weight, the upper slide was pulled horizontally using a thread attached to a hook.
- The time (in seconds) required to separate the two slides was recorded using a stopwatch.
- The following formula was used to calculate the Spreadability:

Spreadability (S) = M×L / T

Where:

- S = Spreadability (g·cm/sec)
- M = Weight tied to the upper slide (500 g)
- L = Length moved by the slide (usually fixed at 7.5 cm)
- T = Time taken to move the slide (in seconds)
- Note: The test was repeated three times, and the average value was taken for final reporting.

(c) Extrudability Test:

Principle:

Extrudability is the measure of the force required to extrude the ointment from a collapsible tube. It reflects the ease of product application and consumer acceptability. A formulation with good extrudability flows smoothly under moderate pressure.

Materials Required:

- Aluminum collapsible or laminated ointment tube (filled with 10–15 g of ointment)
- Clamp or holder
- Weight (usually 1 kg)
- Ruler or scale
- Stopwatch

Procedure:

- A clean, pre-weighed collapsible tube was filled with the herbal ointment and sealed. The tube was then placed between two glass slides and clamped. A standard weight of 1 kg was placed on the slide positioned over the crimped end of the tube for 30 seconds.
- The amount of ointment extruded from the nozzle was collected and measured. The extrudability was calculated as the weight (in grams) of ointment extruded through the nozzle in 30 seconds under standard load.
- Alternatively, extrudability can be expressed using the formula:

Extrudability (E) = Weight of ointment extruded (g) \ Applied force (kg)

Note: The test was performed in triplicate, and the mean value was calculated.

(d) Viscosity Measurement:

Principle:

Viscosity is a measure of a fluid's resistance to flow. It affects the Spreadability, stability, and ease of application of topical formulations. Measuring the viscosity of herbal ointments ensures consistent quality and performance of the product.

Materials Required:

- ❖ Brookfield viscometer (or equivalent rotational viscometer)
- ❖ Suitable spindle (e.g., Spindle No. 64 for semi-solid formulations)
- ❖ Sample container (e.g., 50 mL beaker)
- ❖ Thermometer
- ❖ Herbal ointment sample

Procedure:

- ○ Approximately 30–50 g of the herbal ointment was placed in a clean, dry beaker. The sample was allowed to equilibrate at 25 ± 1°C (room temperature) before measurement. The selected spindle (e.g., Spindle No. 64) was lowered into the sample until it was submerged as per the manufacturer's instructions.

- ○ The viscometer was operated at a suitable rotational speed (e.g., 10, 20, or 50 rpm) and the viscosity was recorded in centipoise (cP) after the reading stabilized. The measurements were repeated at different speeds to observe any non-Newtonian flow behavior. All readings were taken in triplicate to ensure accuracy.

(e) Washability test:

To determine the ease with which the herbal ointment can be removed from the skin surface using water, indicating patient convenience and formulation quality.

Materials Required:

- ❖ Herbal ointment sample
- ❖ Human skin surface or artificial skin model
- ❖ Lukewarm water
- ❖ Mild soap
- ❖ Cotton swabs or soft tissue
- ❖ Stopwatch

Procedure:

- A small quantity of the herbal ointment (approximately 0.5 g) was applied evenly over a marked area (e.g., 2 × 2 cm) on a pre-cleaned skin surface or glass slide. The sample was left undisturbed for 30 minutes to mimic typical contact time.

- After this period, the area was rinsed under lukewarm running water or gently wiped using a cotton swab soaked in water, and the ease of removal was noted.

The washability was graded as follows:

- ❖ Easily washable – completely removed with water
- ❖ Moderately washable – partial residue, removed with mild rubbing
- ❖ Poorly washable – visible residue remains after rinsing
- ❖ The test was performed in triplicate for accuracy, and the average observation was reported.

Stability Study:

- Stability testing was conducted as per ICH Q1A (R2) guidelines. The ointment was stored under the following conditions:

- ○ 40°C ± 2°C / 75% RH ± 5% (accelerated)

- 25°C ± 2°C / 60% RH ± 5% (room temp)
- 4°C ± 2°C (refrigerated)
- Samples were withdrawn at 0, 15, 30 days and evaluated for physical appearance, pH, viscosity, Spreadability, extrudability, and microbial stability.

Preliminary Phytochemical Tests:

Table 2 Observation Table of Phytochemical Test

Test	Observation	Inference
Alkaloids test: **Mayer's test:** 1-2 ml of sample treat with mayer's reagent (few drop)	Cream colour ppt	Presence of alkaloids
Flavanoids test **Alkali test:** 1-2ml of sample +10% aq. sodium hydroxide solution	Red colour	Presence of flavonoids
Tannins test: **Ferric chloride test :** 1-2ml of sample +few drops of ferric chloride solution	Bluish black colour	Presence of tannins
Saponins test : **Froth test:** Small amount of sample+ 5-10ml distilled water shake vigorously for 30sec and left for 20min	Presence of Froth (foam small bubbles)	Presence of saponins
Terpenoids test: **Salowski test:** 1-2ml of sample +2ml of chloroform mix well+3-4 drops of conc. sulfuric acid	Lower chloroform layer of the solution tuns red colour	Presence of terpenoids

RESULT:

Table 3 Results of Evaluation Test

Test	Method/Instrument Used	Result
Organoleptic Properties	Visual and sensory evaluation (Color, Odor, Texture)	Light Cream, herbal odor
pH Determination	Digital pH meter(1%ointmentindistilledwater)	6.2
Spreadability	Slip and drag method	12.5g·cm/sec
Viscosity	Brookfield viscometer	4500cps
Washability	Applied on skin, washed with water	Easily washable
Extrudability	Collapsible tube method	0.9g/cm²
Skin Irritation Test	Patch test on healthy volunteers	No irritation observed

RESULT:

- Organoleptic properties, such as color, odor, and texture, are initially assessed to ensure the product is visually appealing and has a pleasant feel. A well-formulated ointment should have a uniform appearance, a characteristic odor related to its herbal components, and a smooth, non-greasy texture.

- The pH of the ointment is a critical parameter, as it can affect skin compatibility. An ideal pH for a topical formulation is generally slightly acidic, aligning with the skin's natural pH to minimize the risk of irritation.

- Spreadability is evaluated to determine how easily the ointment can be applied to the skin. A good ointment should spread easily and evenly, allowing for uniform coverage of the affected area.

- Viscosity measurements provide information about the ointment's thickness and consistency. The viscosity should be such that the ointment is easy to apply but does not flow excessively, ensuring it remains at the application site for the desired therapeutic effect.

- Washability is an important patient compliance factor. An ointment that is easily washable is preferred, as it can be removed without leaving a greasy residue, enhancing the user experience.

- Extrudability tests assess the ease with which the ointment can be expelled from its container, which is important for convenient application.

- Skin irritation tests are essential to ensure the safety of the formulation. These tests, typically conducted on human volunteers or animal models, should demonstrate that the ointment does not cause significant irritation or adverse reactions.

- Stability studies are carried out to evaluate the ointment's physical and chemical stability over time and under various storage conditions. A stable ointment will maintain its properties, such as appearance, consistency, and efficacy, throughout its shelf life.

- Finally, homogeneity is assessed to ensure that the ointment has a uniform distribution of all its components. This is crucial for consistent dosing and therapeutic effectiveness.

- This type of evaluation ensures that the herbal ointment is not only effective but also safe, stable, and acceptable for patient use.

CONCLUSION:

The successful formulation and evaluation of the herbal ointment incorporating Aloe vera, Neem, and Eco Tamarind extracts demonstrate the potential for developing a stable and user-friendly topical product. The ointment's key physicochemical properties were assessed, revealing a pH of 6.2, which falls within the range considered suitable for dermal applications, thus minimizing the risk of skin irritation. The observed spreadability of 12.5 g·cm/sec indicates that the ointment can be easily applied and distributed across the skin surface, which is a critical factor for patient compliance and effective treatment. The viscosity of 4500 cps suggests a consistency that is neither too thick to hinder application nor too thin to run off the application site, striking a balance for practical use. Furthermore, the ease with which the ointment can be washed off with water is a positive attribute, enhancing user convenience. The extrudability test, with a result of 0.9 g/cm², provides insight into the force

required to expel the ointment from its container, an important consideration for packaging and patient use. Safety is a paramount concern in topical formulations, and the absence of skin irritation observed in the patch tests on healthy volunteers is a significant finding, supporting the ointment's suitability for skin application. The accelerated stability testing, conducted over a period of 1 months under controlled conditions of 40°C ± 2°C and 75% ± 5% relative humidity, revealed no significant changes in the ointment's physical characteristics. This suggests that the formulation is reasonably stable under stress conditions, implying a potential for an acceptable shelf life under normal storage conditions. The uniform and smooth consistency of the ointment, as confirmed by homogeneity assessments, indicates that the components were effectively mixed, contributing to the overall quality of the product. In conclusion, the combined results of the physicochemical evaluations, safety assessment, and stability testing support the potential of this herbal ointment as a viable topical formulation. The incorporation of Aloe vera, Neem, and Eco Tamarind extracts appears to have resulted in a product with favorable attributes for skin application, warranting further investigation into its therapeutic efficacy.

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