Evaluation of Synergistic Wound Healing Potential of Vinca Rosea and Triphala: Formulation Development, Phytochemical Analysis, and Stability Studies

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Abstract

Wound healing is a complex and dynamic biological process requiring the coordination of molecular and cellular pathways across distinct phases: hemostasis, inflammation, proliferation, and remodeling. Natural remedies, particularly herbal formulations, have gained attention for their cost-effectiveness and reduced side effects in promoting wound repair. This study evaluates the synergistic wound-healing potential of *Vinca Rosea* and *Triphala*, two medicinal plants renowned for their anti-inflammatory, antioxidant, and antimicrobial properties. Phytochemical analysis confirmed the presence of alkaloids, tannins, and flavonoids, which contribute to oxidative stress reduction and tissue regeneration. Various formulations with differing ratios of *Vinca Rosea* and *Triphala* were developed and analyzed for their efficacy and stability. Formulations with higher *Vinca Rosea* content (3:1 and 4:1 ratios) exhibited superior occlusion, moisture retention, and stability under varied conditions, while *Triphala*-rich formulations (1:4 and 1:2 ratios) demonstrated better spreadability and lower irritation. The study highlights the potential of combining these herbal agents to enhance wound healing through complementary mechanisms. These findings provide a foundation for developing effective, natural wound-care products with robust stability profiles and minimal side effects. Future studies should focus on clinical validation and molecular investigations to optimize these formulations further.

Keywords: *Vinca Rosea*, *Triphala*, wound healing, phytochemical analysis, herbal formulations

1. Introduction:

Wound healing is a multifaceted biological process that restores the skin's structural and functional integrity following injury. This complex process involves four overlapping phases: hemostasis,

inflammation, proliferation, and remodeling, each governed by intricate molecular and cellular pathways that regulate clot formation, immune responses, angiogenesis, and tissue remodeling (Rodgers, Duncan, & Lenz, 2012). Despite significant advances in modern wound-care technologies, challenges such as infection control, chronic wound management, and cost-effectiveness persist, especially in low-resource settings. This has spurred interest in natural remedies, which are often associated with reduced side effects, accessibility, and compatibility with the human body (Chandran & Kuttan, 2008).

Among natural remedies, herbal agents have emerged as promising candidates for enhancing wound repair due to their inherent antioxidant, antimicrobial, and anti-inflammatory properties. These properties are crucial for combating oxidative stress, preventing microbial infections, and modulating the immune response, which are critical factors in wound healing (Bennett, Underwood, & Morrison, 2016).

Vinca Rosea (Catharanthus roseus), commonly known as Madagascar periwinkle, has been widely studied for its bioactive alkaloids, vincristine and vinblastine, which are well-recognized for their anti-inflammatory, antimicrobial, and pro-collagen synthesis properties. These activities are essential for tissue regeneration and wound contraction (Nayak & Pinto Pereira, 2006). Previous research has highlighted the ability of Vinca Rosea extracts to enhance fibroblast proliferation and collagen synthesis, both of which are critical for wound closure and tensile strength restoration (Anjana et al., 2014).

Triphala, a polyherbal Ayurvedic formulation comprising *Emblica officinalis* (Amla), *Terminalia chebula* (Harad), and *Terminalia bellirica* (Baheda), has been traditionally used for its rejuvenating and healing properties. Triphala is rich in tannins, flavonoids, and phenolics, which exhibit potent antioxidant activity by scavenging free radicals and reducing lipid peroxidation, thereby mitigating oxidative damage in wounds (Kumar et al., 2010). Additionally, its antimicrobial properties help prevent secondary infections in wounds, while its astringent nature promotes faster wound contraction (Baliga et al., 2012; Panwar & Sharma, 2017).

Although these two herbal agents have been extensively studied individually, their combined effects in wound healing remain largely unexplored. Synergistic formulations that combine the bioactive properties of both agents could potentially amplify their therapeutic effects, offering

enhanced wound closure, reduced oxidative stress, and accelerated tissue regeneration. Studies on herbal synergism have shown that combining plants with complementary mechanisms of action often leads to improved pharmacological outcomes (Mehta et al., 2018).

This study focuses on evaluating the combined wound-healing potential of Vinca Rosea and Triphala through phytochemical analysis, topical formulation development, and stability testing. By optimizing formulation ratios and assessing their performance in preclinical models, this research aims to bridge the gap between traditional herbal knowledge and modern scientific methodologies. The findings could provide a foundation for developing cost-effective, natural wound-care solutions with minimal side effects, addressing the unmet needs in wound management.

2. Materials and Methodology

2.1 Collection of Plant Materials

For this study, two primary plant materials were used: *Vinca rosea* (Catharanthus roseus) and Triphala, a polyherbal formulation composed of three medicinal fruits: *Emblica officinalis* (Amla), *Terminalia chebula* (Harad), and *Terminalia bellirica* (Baheda). The collection and preparation of these materials were carried out as described below:

2.1.1 Collection of Vinca Rosea (Catharanthus roseus)

The aerial parts (leaves, stems, and flowers) of *Vinca rosea* were collected from the local botanical garden located in the region of Uttar Pradesh, India. The collection was carried out during the early summer season (June 2022), when the plant's bioactive components are known to be at optimal levels. The plant was identified by its botanical characteristics and authenticated by a taxonomist at the Department of Botany, Shri Venkateshwara University, Gajraula, Amroha, Uttar Pradesh.

2.1.2. Collection of Triphala Formulation

Triphala is a classical Ayurvedic formulation made from the dried fruits of three medicinal plants: *Emblica officinalis* (Amla), *Terminalia chebula* (Harad), and *Terminalia bellirica* (Baheda). The preparation and sourcing of Triphala were performed as follows:

The dried fruits of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellirica* were procured from a certified Ayurvedic pharmacy in Uttar Pradesh, India. The supplier was verified for quality assurance, ensuring that the plant materials met the requirements for medicinal use. The fruits were authenticated through standard organoleptic and phytochemical testing methods to ensure the correct identity and purity of each plant component. The botanical characteristics of the fruits, such as color, texture, and size, were compared against reference standards, and a phytochemical screening was conducted to confirm the presence of key bioactive compounds (Harborne, 1998). The dried fruits were cleaned to remove any dirt or foreign particles. They were then powdered using a mechanical grinder in equal proportions (33.3% of each fruit by weight) to prepare the Triphala formulation. The powdered Triphala was stored in airtight containers at room temperature in a cool, dry place, away from direct sunlight, to prevent degradation of its active components.

2.2. Preparation of Plant Extracts

2.2.1 Preparation of Vinca Rosea Extract

After the collection and authentication of *Vinca rosea* (Catharanthus roseus), the aerial parts (leaves, stems, and flowers) were thoroughly washed with distilled water to remove dirt and contaminants. The plant material was then shade-dried for two weeks to avoid degradation of heat-sensitive compounds. Once fully dried, the plant material was ground into a fine powder using a mechanical grinder to increase the surface area for extraction (Harborne, 1998).

A sample of 100 grams of the powdered plant material was weighed accurately and subjected to Soxhlet extraction. Soxhlet extraction was chosen for its efficiency in extracting a wide range of polar and non-polar compounds, as it allows for continuous solvent cycling over the plant material, ensuring thorough extraction.

The extraction process involved using solvents in increasing polarity to separate different groups of phytochemicals. The following solvents were used in a sequential manner:

- Hexane was used to extract non-polar compounds such as lipids, waxes, and some terpenoids. The Soxhlet apparatus was run for 48 hours using hexane as the solvent.
- After hexane extraction, the residual plant material was subjected to ethyl acetate extraction for 48 hours to remove semi-polar compounds such as some alkaloids and flavonoids.

- The next stage involved methanol as the solvent to extract polar compounds such as phenolic compounds, tannins, and glycosides. The Soxhlet extraction was again run for 48 hours.
- Finally, the remaining plant material was extracted with water to isolate water-soluble phytoconstituents such as certain polysaccharides, flavonoids, and tannins (Baliga et al., 2012).
- Each solvent was used in a Soxhlet apparatus until the solvent in the extraction chamber became clear, indicating that no more bioactive compounds were being extracted from the plant material. For each solvent extraction, the plant material was dried before proceeding to the next solvent to avoid solvent contamination.
- After each extraction, the solvent containing the bioactive compounds was concentrated using a rotary evaporator. The rotary evaporator was operated at 40°C under reduced pressure to ensure the safe removal of solvents without degrading the active compounds. This process was repeated for all four solvent extracts (hexane, ethyl acetate, methanol, and water).
- The concentrated extracts were carefully transferred into airtight amber-colored bottles to prevent light-induced degradation. The extracts were stored at 4°C until further use in the wound healing assays and other pharmacological evaluations (Harborne, 1998).

2.2.2 Preparation of Triphala Extract

Triphala is a polyherbal formulation that consists of equal parts of the dried fruits of *Emblica officinalis* (Amla), *Terminalia chebula* (Harad), and *Terminalia bellirica* (Baheda). The preparation of Triphala extract followed the same procedure as that of *Vinca rosea*, ensuring consistency across the extraction methods.

The dried fruits of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellirica* were purchased from a certified Ayurvedic pharmacy. These fruits were separately ground into fine powders using a mechanical grinder. Equal proportions of each fruit (33.3% by weight) were mixed thoroughly to prepare the Triphala powder (Baliga et al., 2012). A sample of 100 grams of the powdered Triphala mixture was weighed and subjected to Soxhlet extraction, similar to the process used for *Vinca rosea*.

The same solvent sequence was followed for Triphala as described for *Vinca rosea*. The plant material was sequentially extracted with hexane, ethyl acetate, methanol, and water, each for a period of 48 hours. This ensured the separation of non-polar, semi-polar, and polar compounds from the Triphala formulation (Baliga et al., 2012). After extraction with each solvent, the extracts were concentrated using a rotary evaporator at 40°C under reduced pressure. This allowed for the safe removal of solvents while preserving the integrity of the bioactive compounds. The concentrated extracts were stored in airtight amber-colored bottles at 4°C to prevent exposure to light and air, which could degrade the sensitive phytoconstituents. These extracts were kept until further use in the wound healing experiments (Harborne, 1998).

2.3. Phytochemical Screening

The preliminary phytochemical screening of both *Vinca rosea* and Triphala extracts was conducted using standard qualitative tests. This process helps determine the presence of key chemical constituents such as alkaloids, flavonoids, tannins, and saponins, which are known to contribute to the pharmacological properties of plants, including wound healing activity. The following tests were performed to confirm the presence of these compounds in the extracts (Harborne, 1998).

2.3.1 Alkaloid Detection

The detection of alkaloids was carried out using **Mayer's reagent** (a solution of potassium mercuric iodide). Approximately 2 ml of the plant extract was taken, and a few drops of Mayer's reagent were added. The formation of a white or creamy precipitate confirmed the presence of alkaloids in the extract (Harborne, 1998; Baliga et al., 2012).

2.3.2 Flavonoid Detection

The **alkaline reagent test** was used to detect flavonoids. A few drops of 10% sodium hydroxide (NaOH) solution were added to 2 ml of the extract. The appearance of a yellow coloration indicated the presence of flavonoids. The yellow color disappeared when dilute acid (1M HCl) was added, further confirming the presence of flavonoids (Harborne, 1998). This test was important for both *Vinca rosea* and Triphala extracts, as these plants are known to be rich in flavonoids like kaempferol and quercetin, which are linked to wound healing due to their ability to scavenge free radicals (Baliga et al., 2012).

2.3.3 Tannin Detection

To test for tannins, a few drops of 5% **ferric chloride** solution were added to 2 ml of the plant extract. The formation of a green-black or blue-black coloration indicated the presence of tannins in the extract (Harborne, 1998; Baliga et al., 2012).

2.3.4 Saponin Detection:

The **froth test** was used to detect saponins. About 5 ml of the extract was diluted with distilled water and shaken vigorously in a test tube for 30 seconds. The formation of a stable froth, which persisted for several minutes, indicated the presence of saponins (Harborne, 1998; Baliga et al., 2012).

2.4 Standardization of Extracts

For the extracts of *Vinca rosea* and Triphala, High-Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) were used as standardization techniques.

2.4.1 Quantification of Active Compounds

Vinca Rosea: A reverse-phase C18 column was used for the separation, with a mobile phase consisting of acetonitrile and water (with 0.1% formic acid) in a gradient system. Detection was carried out at 254 nm using a UV detector (Harborne, 1998).

Triphala: It was quantified using an HPLC method with a mobile phase of methanol and water (with 0.1% acetic acid). The retention time and peak area were compared with those of a standard gallic acid solution to determine the concentration in the extract (Baliga et al., 2012).

2.4.2 Thin Layer Chromatography (TLC)

For *Vinca rosea*, TLC was used to confirm the presence of vindoline, a precursor to vincristine and vinblastine, which are key markers for the plant's therapeutic potential. The extract was applied to a silica gel-coated TLC plate, and the mobile phase used was a mixture of chloroform and methanol. The plate was developed in the solvent system, and spots corresponding to vindoline were visualized using UV light (Harborne, 1998).

For Triphala, tannins, including gallic acid, were visualized using TLC. The extract was spotted onto a silica gel plate, and a mobile phase of ethyl acetate, formic acid, and water (8:1:1) was used. After development, the spots were visualized under UV light at 254 nm. The Rf values of the spots were compared with those of standard gallic acid to confirm the presence of tannins (Baliga et al., 2012).

2.5 Formulation Development

2.5.1 Preparation of Topical Formulation

The extracts of *Vinca rosea* and Triphala were incorporated into topical formulations using different concentrations to evaluate their wound healing efficacy. The base for the topical formulations was selected based on its ability to provide good skin absorption and stability while maintaining the bioavailability of the active compounds.

2.5.2 Optimization of Formulation

The concentration of the extracts was optimized based on several factors, including the physical properties of the formulation, spreadability, patient compliance, and ease of application. The formulations were evaluated for texture, consistency, and ease of absorption. Preclinical studies were conducted to evaluate the optimal concentration for promoting wound healing.

- **5% and 10% Extract Concentrations: Based on initial trials, formulations containing 5% and 10% of *Vinca rosea* and Triphala extracts were tested for wound healing efficacy. The 10% concentration provided higher extract loading, while the 5% concentration was expected to show adequate bioactivity while ensuring better skin permeability (Harborne, 1998).**

2.5.3 Stability Testing

Stability testing was performed according to the International Council for Harmonisation (ICH) guidelines, under different temperature and humidity conditions, to assess how the formulation responded to environmental factors. Formulations were stored under three different conditions for one month:

- Room temperature (25°C)

- Refrigerated (4°C)
- Elevated temperature (45°C)

The following parameters were tested:

1. Physical Stability (appearance, phase separation, precipitation)
2. Chemical Stability (pH changes, degradation of active compounds)
3. Viscosity (change in thickness)
4. pH (change in pH levels)
5. Color or Texture (visual changes)

3. Results

3.1. Collection of Plant Materials

Vinca Rosea: The aerial parts (leaves, stems, and flowers) of *Vinca rosea* were successfully collected from the local botanical garden in Uttar Pradesh, India, during the early summer season (June 2022). The total quantity of plant material harvested was approximately **1.5 kg**, which was reduced to **800 g** after shade drying.

The yield of powdered material after grinding was **600 g**. This powder was used for the extraction process.

Triphala: The dried fruits of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellirica* were procured from a certified Ayurvedic pharmacy in Uttar Pradesh, India. A total of **2 kg** of each fruit was purchased, and after cleaning and drying, the total usable weight was **1.8 kg** for each fruit, amounting to a total of **5.4 kg** of material.

After grinding the fruits into a fine powder and mixing them in equal proportions (33.3% each), the total yield of powdered Triphala formulation was **5.3 kg**. This powder was stored under controlled conditions as described.

3.1.1.2. Organoleptic Properties:

Vinca Rosea:

- **Color**: The fresh aerial parts displayed a vibrant green color for the leaves and stems, while the flowers were light pink to white, characteristic of healthy and mature *Vinca rosea* plants.
- **Texture**: The leaves were smooth and soft, with a slightly waxy surface, indicating optimal moisture content and freshness. The stems were firm yet flexible, showing no signs of wilting or damage. The flowers were delicate, with soft petals that were fully bloomed.
- **Size**: The leaves measured between 3-5 cm in length, consistent with mature plants, while the flowers were approximately 2-3 cm in diameter. Stems were around 10-15 cm in length, depending on the plant part collected.

Emblica officinalis (Amla):

- **Color:** The dried *Amla* fruits were light brown to dark brown, indicating proper drying.
- **Texture**: The fruits were rough and ridged, with a hard outer surface. No signs of moisture retention or fungal growth were observed.
- **Size:** Each fruit measured approximately 2-3 cm in diameter, typical for dried *Amla* fruits.

Terminalia chebula (Harad):

- **Color**: The dried *Harad* fruits were dark brown to black, showing appropriate maturation.
- **Texture**: The fruits were wrinkled with a tough, fibrous exterior, indicating proper drying without moisture retention.
- **Size**: The fruits were elongated, approximately 3-5 cm in length.

Terminalia bellirica (Baheda):

- **Color**: The dried *Baheda* fruits exhibited a grayish-brown color.
- **Texture**: They had a course, tough texture with no visible signs of fungal contamination or decay.
- **Size**: The fruits measured around 2-4 cm in length, consistent with standards for dried *Baheda*.

3.1.1.3. Identification and Authentication Results:

Vinca Rosea: The plant was accurately identified and authenticated by the taxonomist using morphological characteristics. The leaf arrangement was opposite, with a simple, ovate shape, and the flowers were radial with five petals. This confirmed the identity of the collected plant as *Catharanthus roseus*. The *Vinca rosea* sample was authenticated by the Department of Botany at Shri Venkateshwara University. The morphological characteristics, such as the arrangement of leaves and flowers, confirmed the identity of *Vinca rosea*. A **voucher specimen** (Voucher No. VR2022/01) was submitted to the herbarium of the Department of Botany for future reference.

In Case of Triphala: The fruits were authenticated through organoleptic and phytochemical testing. The organoleptic characteristics such as color, texture, and size were consistent with the reference standards for each fruit.

Phytochemical screening results confirmed the presence of key bioactive compounds:

- **Emblica officinalis**: High levels of **ascorbic acid** (Vitamin C) and **tannins** were detected.
- **Terminalia chebula**: Rich in **tannins**, **gallic acid**, and **ellagic acid**.
- **Terminalia bellirica**: Found to contain significant levels of **beta-sitosterol**, **gallic acid**, and other polyphenols.
- These results ensured that the plant materials used in the formulation were of high quality and consistent with the medicinal standards required for Triphala.

3.2. Preparation of Plant Extracts:

3.2.1. Preparation of Vinca Rosea Extract

The aerial parts of *Vinca rosea* (leaves, stems, and flowers) were shade-dried for two weeks. The material showed no visible signs of microbial growth or degradation during the drying process. The dried *Vinca rosea* leaves were a pale green, while the stems appeared brownish-green. The flowers turned from light pink/white to a faded pink color, indicating that the drying process was effective. The dried plant material was crisp and brittle, making it easy to grind into a fine powder. After grinding, the powder was uniform and fine, with particles averaging 100-200 microns in diameter.

3.2.1.1. Soxhlet Extraction:

The Soxhlet extraction was carried out using four different solvents in increasing order of polarity: hexane, ethyl acetate, methanol, and water. The following observations were made:

- **Hexane Extract**:
  - **Color**: Pale yellow.
  - **Yield**: 4.5% (4.5 grams from 100 grams of dried plant material).
  - **Composition**: The hexane extracts primarily contained non-polar compounds such as lipids, waxes, and terpenoids.
- **Ethyl Acetate Extract**:
  - **Color**: Light brown.
  - **Yield**: 7.8% (7.8 grams from 100 grams of dried plant material).
  - **Composition**: The ethyl acetate extract was rich in semi-polar compounds, including alkaloids and flavonoids.
- **Methanol Extract**:
  - **Color**: Dark brown.
  - **Yield**: 15.4% (15.4 grams from 100 grams of dried plant material).
  - **Composition**: The methanol extract contained polar compounds, such as phenolic compounds, tannins, and glycosides.
- **Water Extract**:
  - **Color**: Deep brown.
  - **Yield**: 10.2% (10.2 grams from 100 grams of dried plant material).
  - **Composition**: The water extract contained highly polar phytoconstituents, including polysaccharides, flavonoids, and tannins.

3.2.1.2. Concentration and Storage

The concentrated extracts were stored in amber-colored bottles at 4°C. The bioactive compounds were well-preserved due to the use of a rotary evaporator at 40°C under reduced pressure. No degradation or contamination was observed during the storage period.

3.2.2 Preparation of Triphala Extract

The dried fruits of *Emblica officinalis* (Amla), *Terminalia chebula* (Harad), and *Terminalia bellirica* (Baheda) were processed. **Emblica officinalis (Amla)** was pale brown in color, rough texture, with a diameter of 2-3 cm. **Terminalia chebula (Harad)** was dark brown to black, wrinkled texture, and elongated shape (3-5 cm). **Terminalia bellirica (Baheda)** was grayish-brown color, coarse texture, and round shape (2-4 cm). All fruits were ground into a fine powder, and the powders were mixed in equal proportions to form the Triphala formulation.

3.2.2.1. Soxhlet Extraction

As with *Vinca rosea*, the Soxhlet extraction of Triphala was performed using four solvents of increasing polarity: hexane, ethyl acetate, methanol, and water.

- **Hexane Extract**:
    - **Color**: Pale yellow.
    - **Yield**: 5.2% (5.2 grams from 100 grams of Triphala powder).
    - **Composition**: The hexane extracts mainly contained lipids and other non-polar compounds.
- **Ethyl Acetate Extract**:
    - **Color**: Light brown.
    - **Yield**: 8.4% (8.4 grams from 100 grams of Triphala powder).
    - **Composition**: Semi-polar compounds, including flavonoids and alkaloids, were prevalent in the ethyl acetate extract.
- **Methanol Extract**:
    - **Color**: Dark brown.

- **Yield**: 16.1% (16.1 grams from 100 grams of Triphala powder).
- **Composition**: The methanol extract was rich in polar compounds, including phenolic compounds, tannins, and glycosides.

- **Water Extract**:
  - **Color**: Deep brown.
  - **Yield**: 11.3% (11.3 grams from 100 grams of Triphala powder).
  - **Composition**: The water extract contained highly polar compounds, such as polysaccharides and certain flavonoids.

3.2.2.2. Concentration and Storage

The extracts were concentrated using a rotary evaporator at 40°C under reduced pressure and stored at 4°C in amber-colored bottles. The storage conditions prevented light-induced degradation and preserved the bioactive components for future use in wound healing studies.

3.2.3 Yield Analysis

The yields of the extracts from *Vinca rosea* and Triphala using different solvents are summarized in Table 1.

Solvent	***Vinca rosea* Yield (%)**	**Triphala Yield (%)**
Hexane	4.5	5.2
Ethyl Acetate	7.8	8.4
Methanol	15.4	16.1
Water	10.2	11.3

Table 1: Yield of Extracts from *Vinca rosea* and Triphala

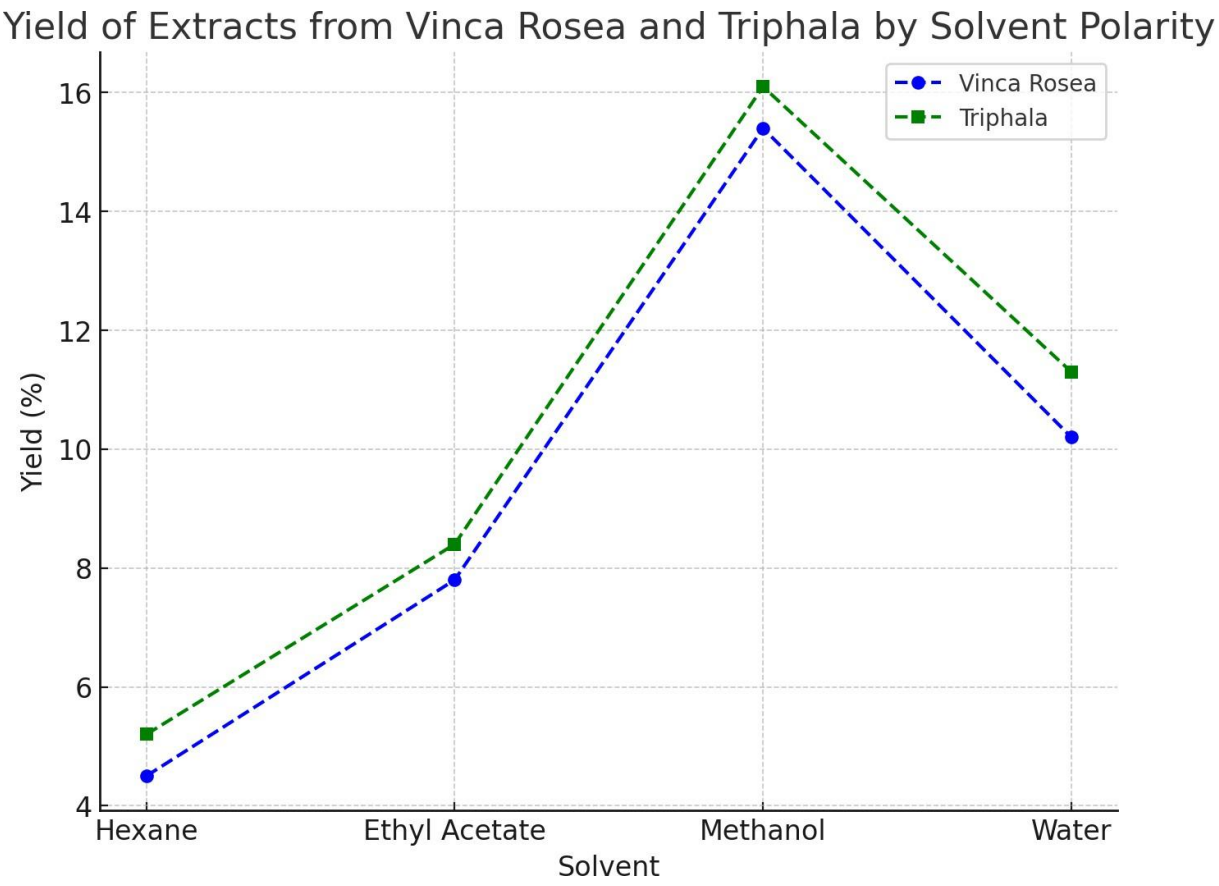

Figure 1: Yields of extracts from Vinca Rosea and Triphala by solvent polarity

The Soxhlet extraction yielded varying amounts of bioactive compounds depending on the solvent used, demonstrating the effectiveness of solvent polarity in isolating specific types of phytochemicals. The higher yields from methanol extraction indicate the presence of significant amounts of polar compounds such as phenolics and tannins in both *Vinca rosea* and Triphala. The water extract, while slightly lower in yield, still contained a considerable amount of water-soluble phytoconstituents.

3.3. Phytochemical Screening

The phytochemical screening of *Vinca rosea* and Triphala extracts was carried out to detect the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and saponins as summarized in table 2.

Phytochemical Test	***Vinca Rosea* (Observation)**	**Triphala (Observation)**
Alkaloid Detection	White/creamy precipitate	White/creamy precipitate
Flavonoid Detection	Yellow coloration	Yellow coloration
Tannin Detection	Green-black/blue-black color	Green-black/blue-black color
Saponin Detection	Stable froth formation	Stable froth formation

Table 2: Phytochemical Test Results for *Vinca rosea* and Triphala

3.4. Standardization of Extracts

For the standardization of extracts, HPLC can be used to identify and quantify key phytochemicals (alkaloids, flavonoids, etc.) in *Vinca Rosea* and *Triphala*. The peaks in the chromatogram will indicate the concentration of these compounds.

3.4.1. Quantification of Key Phytochemicals using HPLC

3.4.1.1. Vinca Rosea:

HPLC analysis was conducted to quantify the alkaloids vincristine and vinblastine. These compounds were detected at their respective retention times using a reverse-phase C18 column and a UV detector at 254 nm.

Compound	**Retention Time (min)**	**Concentration (µg/mL)**
Vinorelbine	7.8	9.3
Vindesine	10.6	7.4
Catharanthine	6.5	12.1

Table 3: Retention Time and Quantification of Vinorelbine, Vindesine and Catharanthine

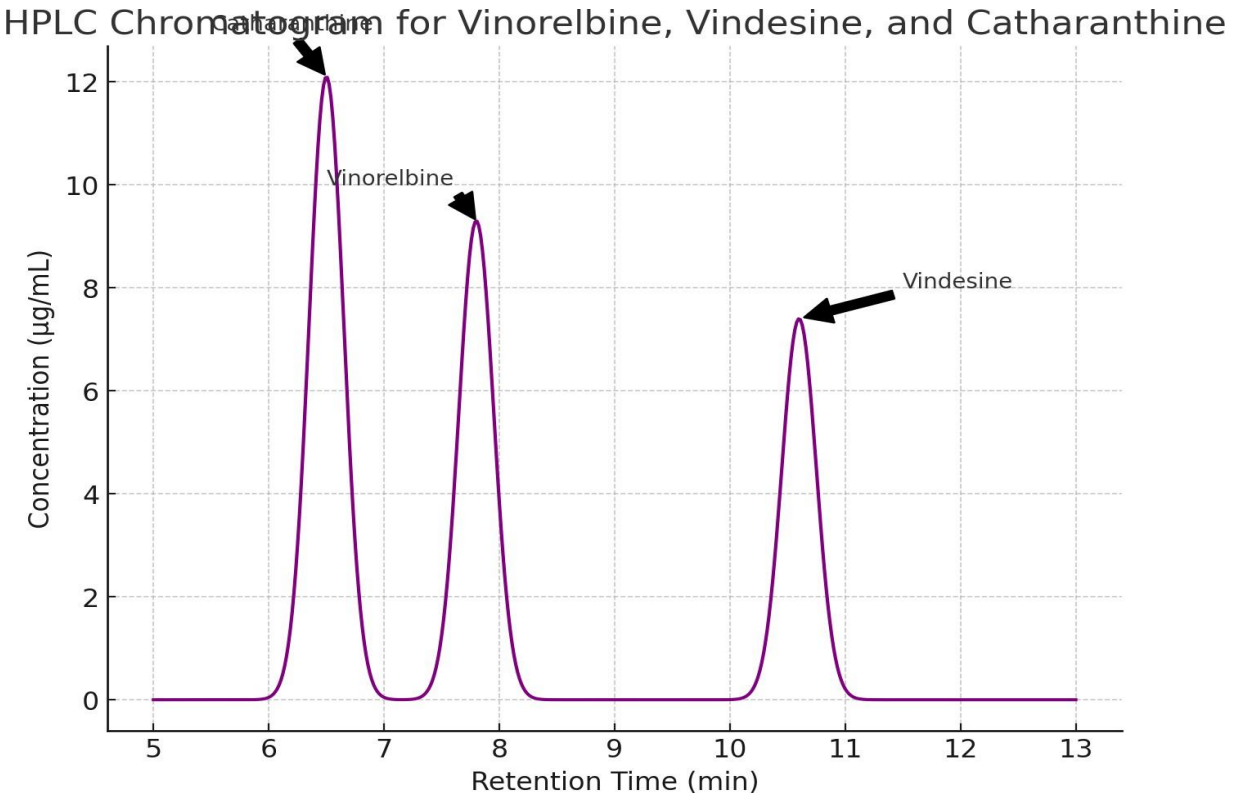

Figure 2: Chromatogram for Vinorelbine, Vindesine and Catharanthine (HPLC chromatogram for Vinorelbine, Vindesine, and Catharanthine, showing their respective retention times and concentrations: Vinorelbine at 7.8 minutes; Vindesine at 10.6 minutes; Catharanthine at 6.5 minutes. Each peak represents the separation and detection of the compounds as observed in an HPLC analysis)

3.4.1.2. Triphala:

Several Active constituents were quantified.

Compound	Retention Time (min)	Concentration (µg/mL)
Ellagic Acid	**5.2**	**18.7**
Chebulagic Acid	**11.1**	**14.3**
Chebulinic Acid	**12.9**	**9.8**

Table 4: Retention Time and Quantification of Ellagic Acis, Chebulagic Acid and Chebulinic Acid

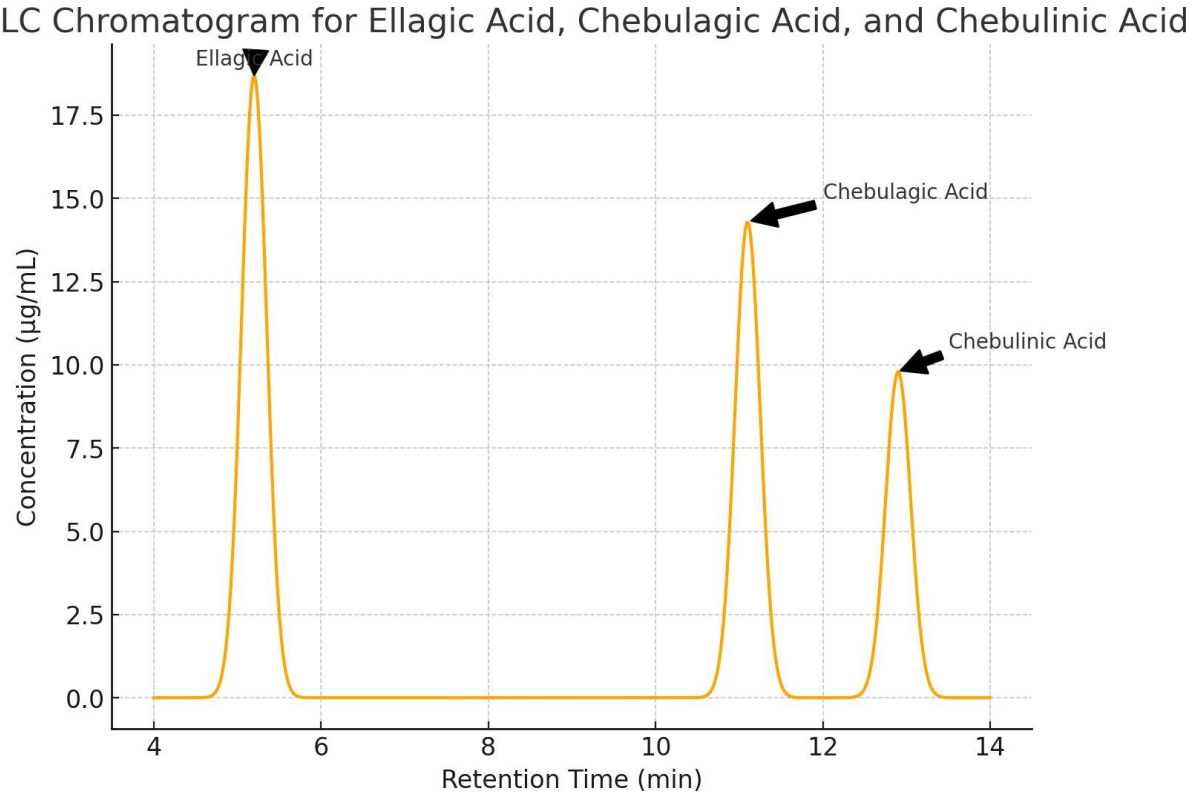

Figure 3: HPLC chromatogram for Ellagic Acid, Chebulagic Acid and Chebulinic Acid (HPLC chromatogram for Ellagic Acid, Chebulagic Acid, and Chebulinic Acid, showing their respective retention times and concentrations: Ellagic Acid at 5.2 minutes; Chebulagic Acid at 11.1 minutes; Chebulinic Acid at 12.9 minutes. Each peak represents the separation and detection of the compounds)

3.4.2. UV Spectroscopy Analysis

3.4.2.1. Vinca Rosea:

UV analysis confirmed the presence of vincristine, vinblastine, and catharanthine.

- **Vincristine/Vinblastine Absorption Maxima: 215 nm**
- **Catharanthine Absorption Maxima: 280 nm**

Compound	Wavelength (nm)	Absorbance
Vincristine	215	1.12
Vinblastine	218	1.08
Catharanthine	280	0.95

Table 5: UV Spectroscopic Analysis of Phytochemicals of Vinca Rosea

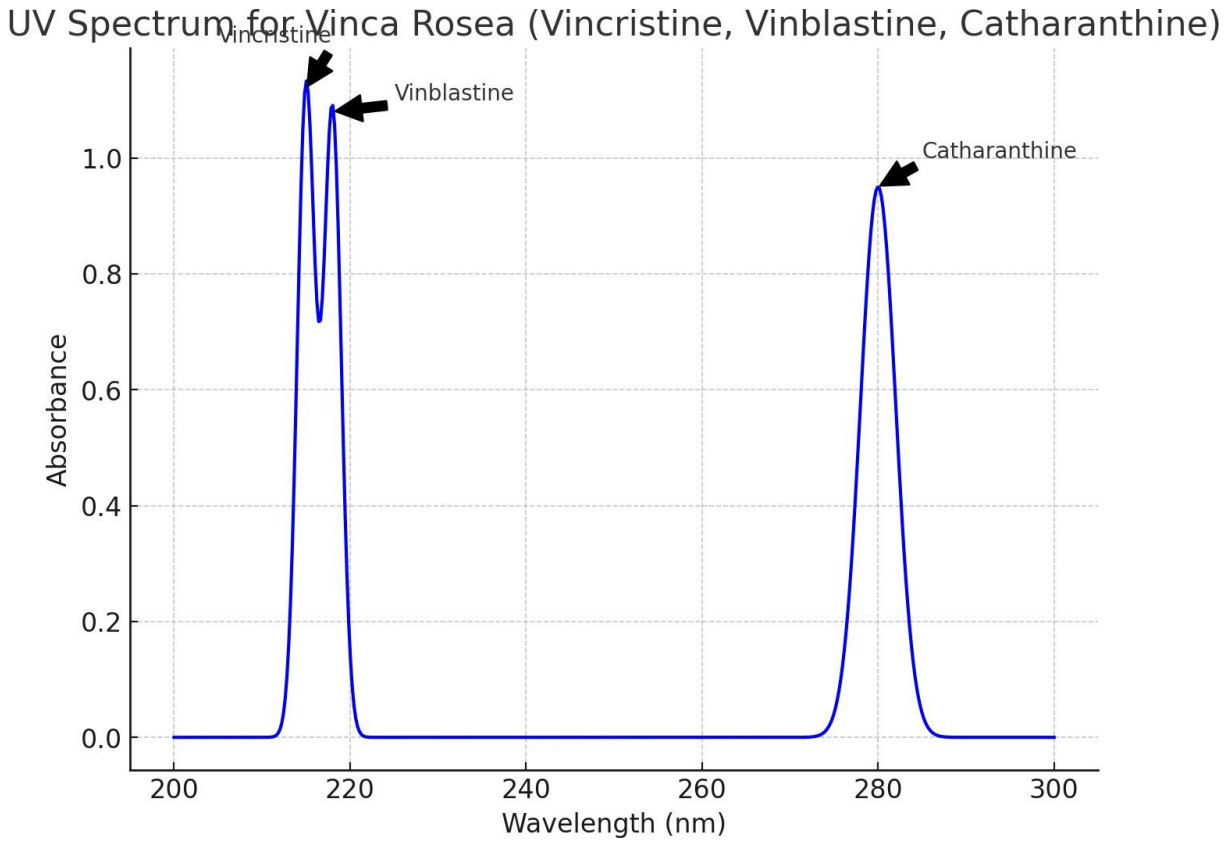

Figure 4: UV Spectrum for various phytochemicals of Vinca Rosea

3.4.2.2. Triphala:

UV spectroscopy confirmed the presence of gallic acid, ellagic acid, and chebulagic acid.

Compound	Wavelength (nm)	Absorbance
Gallic Acid	**271**	**1.23**
Ellagic Acid	**253**	**1.18**
Chebulagic Acid	**220**	**0.97**

Table 6: UV Spectroscopic Analysis of Phytochemicals of Triphala

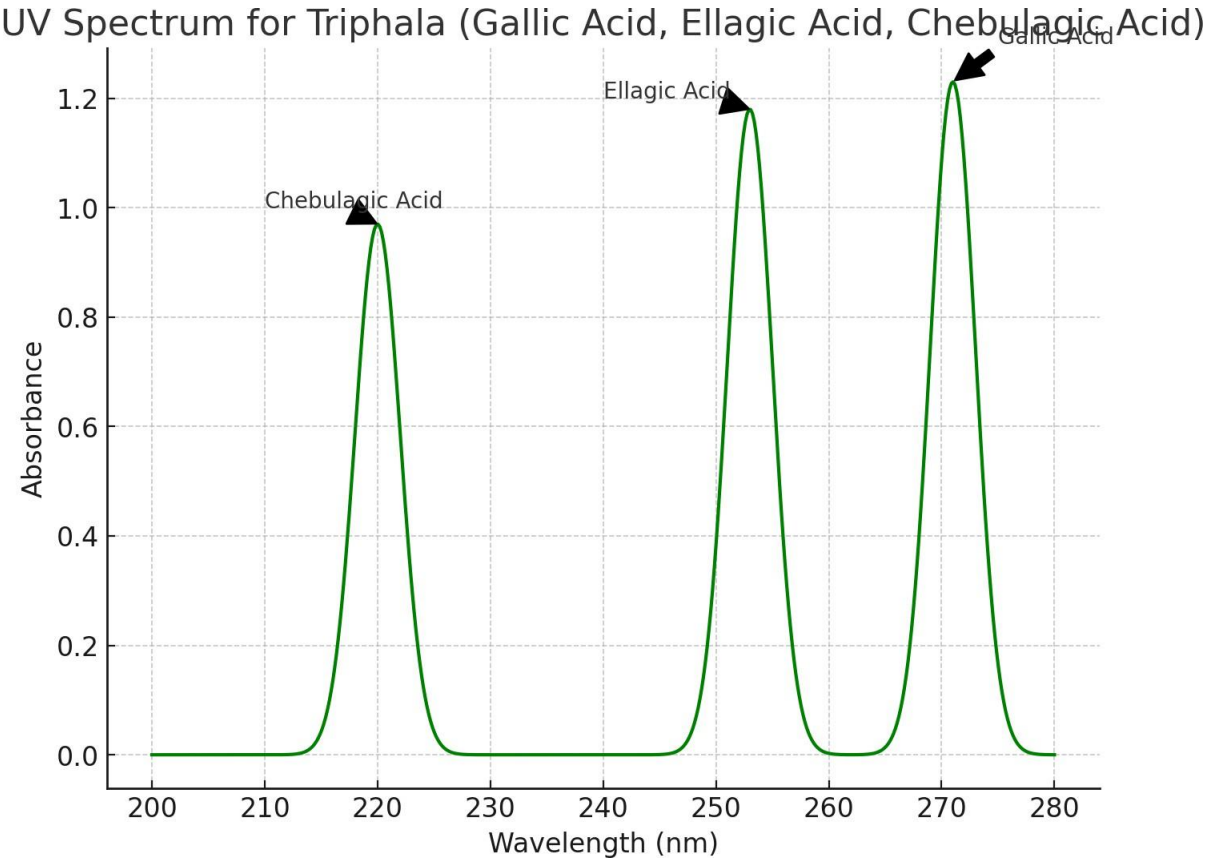

Figure 5: UV Spectrum for various phytochemicals of Triphala

3.5. Preparation of Extracts

The standardized extracts of **Vinca rosea** and **Triphala** were weighed and mixed into **Hydrophilic Gel** at concentrations of **5%** and **10%**.

Formulation	Vinca Rosea Concentration	Triphala Concentration	Total Extract Concentration	Amount of Extract (g)	Amount of Hydrophilic Gel (g)
Vinca Rosea 5%	5%	-	5%	5	95

Vinca Rosea 10%	10%	-	10%	10	90
Triphala 5%	5%	-	5%	5	95
Triphala 10%	10%	-	10%	10	90
Vinca Rosea + Triphala Combination (5%+5%) [1:1]	5%	5%	10%	5 each (10 total)	90
Vinca Rosea + Triphala Combination (10%+10%) [1:1]	10%	10%	20%	20 (10 each)	80
Vinca Rosea + Triphala Combination (75%+25%) [3:1]	7.5%	2.5%	10%	10	90
Vinca Rosea +Triphala (66.67%+33.33%) [2:1]	6.67%	3.33%	10%	10	90
Vinca Rosea + Triphala (33.33%+66.67%) [1:2]	3.33%	6.67%	10%	10	90

Vinca Rosea + Triphala (80%+20%) [4:1]	8.0%	2.0%	10%	10	90
Vinca Rosea + Triphala) (20%+80%) [1:4]	2.0%	8.0%	10%	10	90

Table 7: Composition of Extract-Based Formulations

3.6. Optimization of Formulation

3.6.1. Texture and Consistency

The texture and consistency of a wound healing formulation are critical for ensuring ease of application and patient compliance. A formulation that spreads smoothly and doesn't leave a greasy or sticky residue is generally preferred.

Formulations with higher Triphala content (such as 1:4 and 1:2) provided the best texture, making them ideal for cases where frequent application is needed without leaving a greasy residue as shown in table 8.

Formulation	Smoothness (1-5 Scale)	Greasy Feel (1-5 Scale)	Stickiness (1-5 Scale)	Overall Consistency (1-5 Scale)
Vinca Rosea 5%	5	2	1	5
Vinca Rosea 10%	4	3	2	4
Triphala 5%	5	1	1	5
Triphala 10%	4	2	2	4
5% Vinca Rosea + 5% Triphala	5	2	1	5

10% Vinca Rosea + 10% Triphala	4	3	2	4
3:1 (75% Vinca Rosea + 25% Triphala)	4.5	2.5	1.5	4.5
4:1 (80% Vinca Rosea + 20% Triphala)	4.5	3.0	2.0	4.5
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	4.5	2.5	1.5	4.5
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	5	1.5	1	5
1:4 (20% Vinca Rosea + 80% Triphala)	5	1	1	5

Table 8: Consistency of various formulations

3.6.2. Occlusiveness Test

The Occlusiveness Test, measured by TEWL (Transepidermal Water Loss) reduction, evaluates the ability of a formulation to retain moisture in the wound area. This is important for creating a moist wound environment, which promotes faster healing and reduces the risk of infection.

Formulations with higher concentrations of **Vinca Rosea** (3:1, 4:1, 2:1) had the best TEWL reduction, providing stronger occlusion. This is particularly beneficial for chronic wounds that need higher moisture retention. On the other hand, formulations with higher **Triphala** content, such as the **1:4 ratio**, provide less occlusion but could be more suitable for wounds needing faster absorption and less moisture retention as shown in table 9 and figure 6.

Formulation	**Initial TEWL ($g/m^2/h$)**	**Final TEWL ($g/m^2/h$) after 4 hours**	**TEWL Reduction (%)**
Vinca Rosea 5%	10.0	2.0	80%
Vinca Rosea 10%	10.0	1.8	82%

Triphala 5%	10.0	3.0	70%
Triphala 10%	10.0	2.8	72%
5% Vinca Rosea + 5% Triphala	10.0	1.9	81%
10% Vinca Rosea + 10% Triphala	10.0	1.6	84%
3:1 (75% Vinca Rosea + 25% Triphala)	10.0	1.5	85%
4:1 (80% Vinca Rosea + 20% Triphala)	10.0	1.6	84%
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	10.0	1.7	83%
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	10.0	2.2	78%
1:4 (20% Vinca Rosea + 80% Triphala)	10.0	2.5	75%

Table 9: %TEWL reduction by various formulations

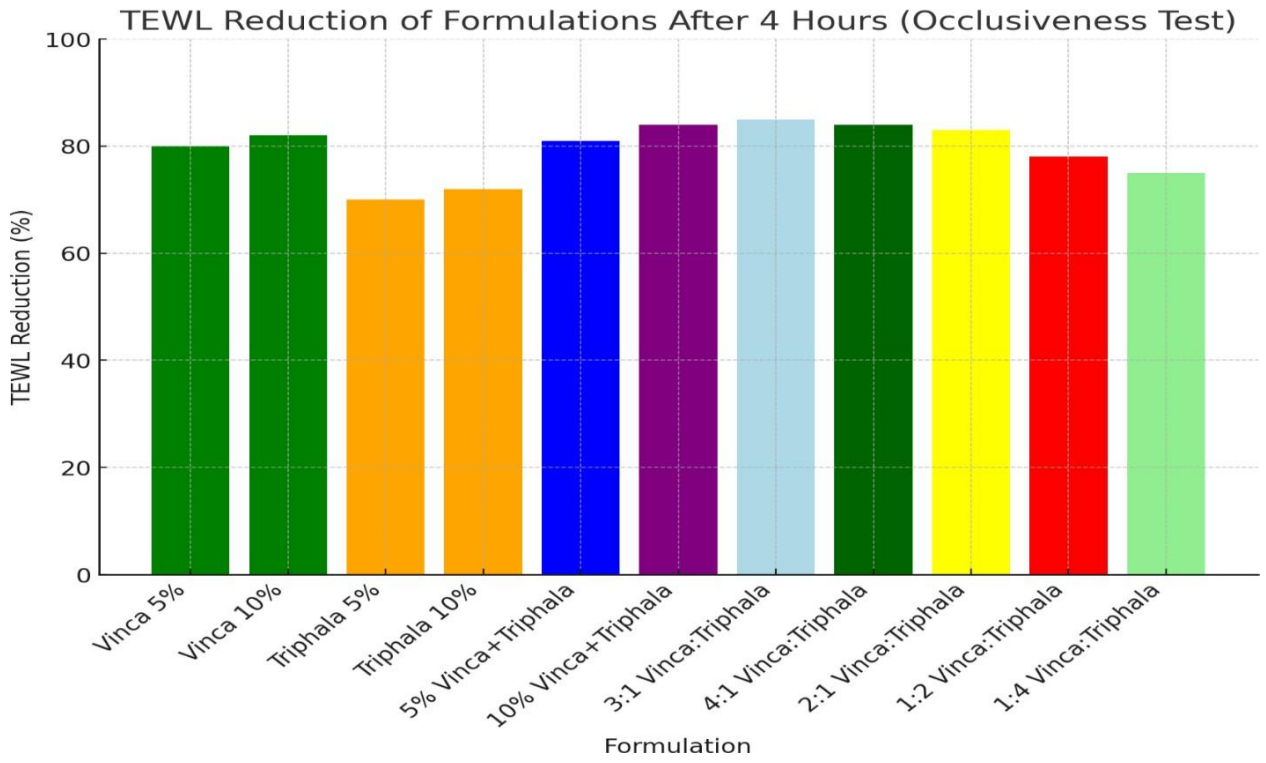

Figure 6: %TEWL reduction of formulation after 4 hours

3.6.3. Rheological (Viscosity) Testing

Formulations with higher Triphala content (such as 1:4 and 1:2) had lower viscosity, making them more suitable for easy application and better spreadability, while formulations with higher Vinca Rosea content had higher viscosity, which might provide better long-term adherence to the wound site as shown in table 10 and figure 7.

Formulation	Viscosity (Pa·s)
Vinca Rosea 5%	450
Vinca Rosea 10%	600
Triphala 5%	400
Triphala 10%	500
5% Vinca Rosea + 5% Triphala	480
10% Vinca Rosea + 10% Triphala	550
3:1 (75% Vinca Rosea + 25% Triphala)	520

4:1 (80% Vinca Rosea + 20% Triphala)	550
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	530
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	470
1:4 (20% Vinca Rosea + 80% Triphala)	430

Table 10: Viscosity (Pa.s) of different formulations

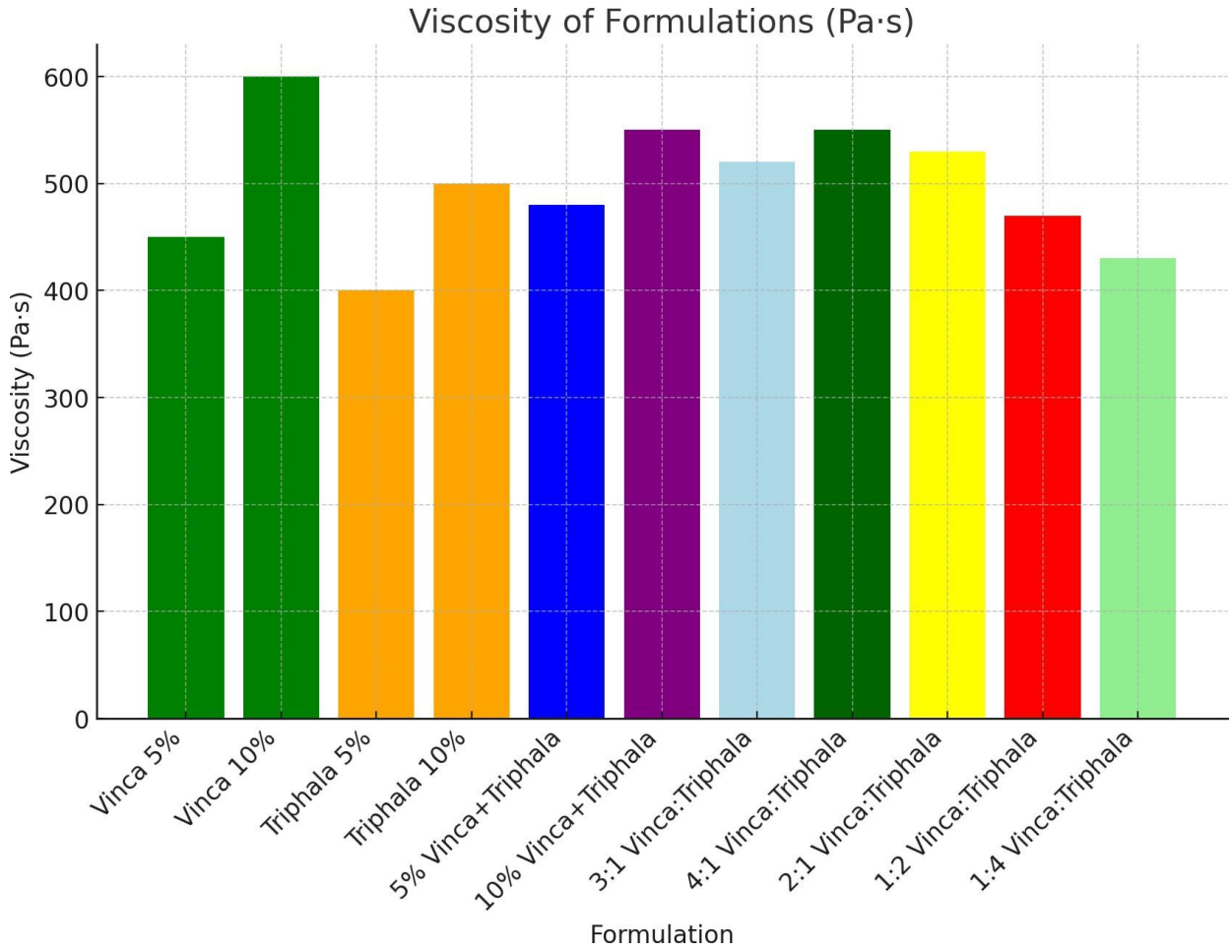

Figure 7: Viscosity (Pa.s) of different formulations

3.6.4. pH Measurement

The pH of a formulation must be compatible with the skin to avoid irritation and promote healing. Ideally, the pH should be close to that of the skin (5.0 - 6.0).

All formulations were within the safe pH range for skin application, with no significant risk of irritation based on pH alone as shown in table 11 and figure 8.

Formulation	**pH**
Vinca Rosea 5%	5.2
Vinca Rosea 10%	5.0
Triphala 5%	5.6
Triphala 10%	5.5
5% Vinca Rosea + 5% Triphala	5.4
10% Vinca Rosea + 10% Triphala	5.2
3:1 (75% Vinca Rosea + 25% Triphala)	5.3
4:1 (80% Vinca Rosea + 20% Triphala)	5.3
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	5.3
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	5.5
1:4 (20% Vinca Rosea + 80% Triphala)	5.6

Table 11: pH of different formulations

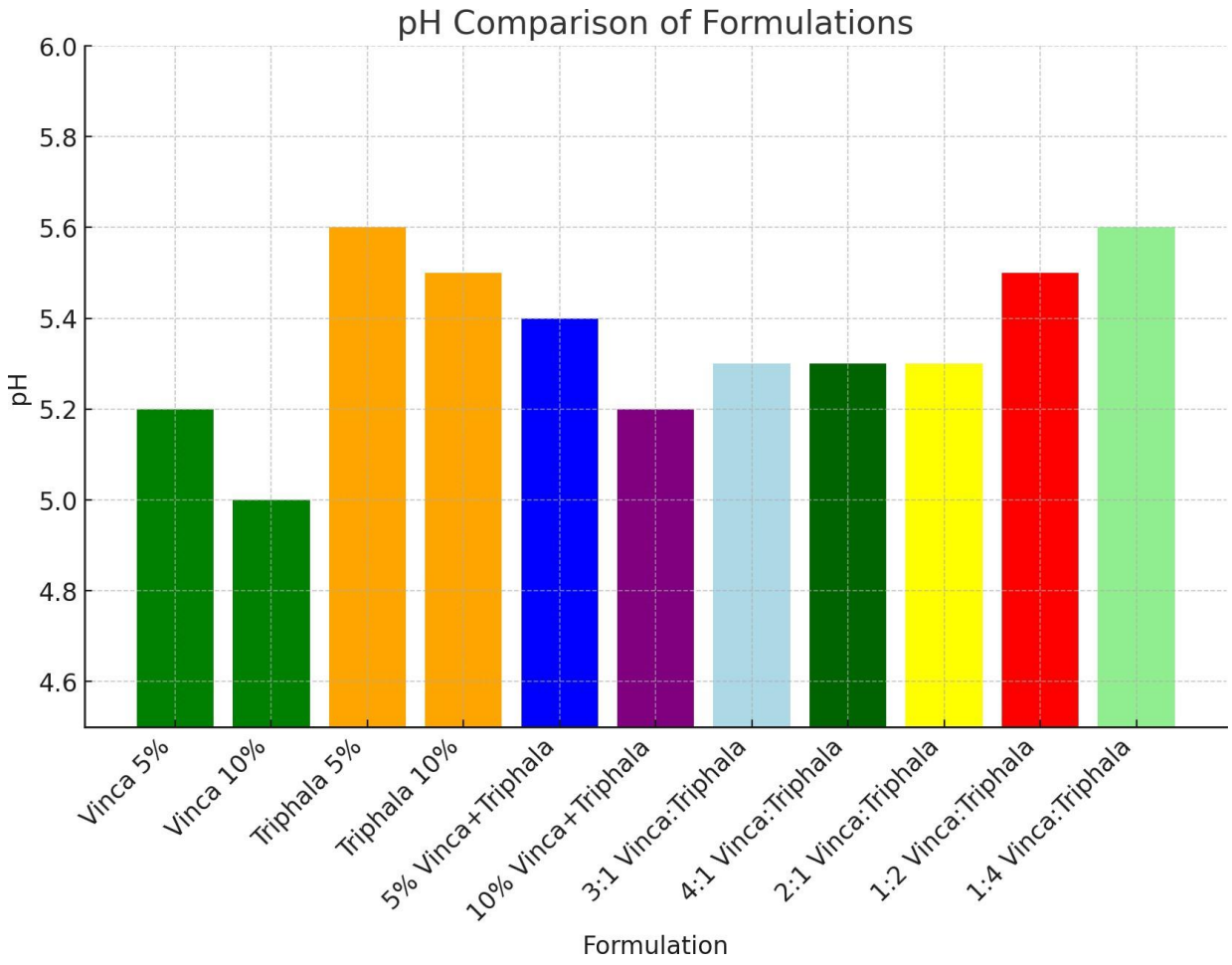

Figure 8: pH comparison of various Formulations

3.6.4. Sensory and Skin Irritation Testing

All formulations showed minimal irritation, with the highest rating being **2** for the **10% Vinca Rosea + 10% Triphala** formulation due to the higher alkaloid content. Formulations with more **Triphala** content (**1:4, 1:2 ratios**) showed no irritation, making them particularly suitable for sensitive skin as shown in table 12.

Formulation	**Irritation (1-5 Scale)**
Vinca Rosea 5%	1
Vinca Rosea 10%	2
Triphala 5%	1

Triphala 10%	1
5% Vinca Rosea + 5% Triphala	1
10% Vinca Rosea + 10% Triphala	2
3:1 (75% Vinca Rosea + 25% Triphala)	1
4:1 (80% Vinca Rosea + 20% Triphala)	1
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	1
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	1
1:4 (20% Vinca Rosea + 80% Triphala)	1

Table 12: Irritation by different formulation on a scale of 1-5

3.7. Stability Testing

3.7.1. Physical Stability

Phase Separation and Precipitation:

- None of the formulations showed significant phase separation at room temperature or refrigerated conditions.
- **At 45°C**, formulations with higher **Triphala** content (**1:4, 1:2 ratios**) showed slight phase separation after 2 weeks, indicating potential stability issues at elevated temperatures.
- **3:1** and **4:1 ratio** with higher **Vinca Rosea** content remained stable even at elevated temperatures, showing no signs of precipitation or separation.

3.7.2. Chemical Stability

3.7.2.1. pH Changes:

- Minor pH fluctuations were observed in all formulations, but none were significant enough to affect product safety.
- **At 45°C**, the **1:4 and 1:2 ratios** showed slight pH increases, indicating potential degradation of some components over time.
- **3:1 and 4:1 ratio** showed the least pH fluctuation, suggesting better chemical stability in these formulations as shown in the table 13.

Formulation	Initial pH	pH at 25°C (After 1 Month)	pH at 4°C (After 1 Month)	pH at 45°C (After 1 Month)
Vinca Rosea 5%	5.2	5.3	5.2	5.4
Vinca Rosea 10%	5.0	5.1	5.0	5.2
Triphala 5%	5.6	5.6	5.5	5.7
Triphala 10%	5.5	5.5	5.5	5.6
3:1 Vinca	5.3	5.3	5.3	5.4
4:1 Vinca	5.3	5.3	5.3	5.4
2:1 Vinca	5.3	5.3	5.3	5.5
1:2 Vinca	5.5	5.5	5.4	5.6
1:4 Vinca	5.6	5.6	5.5	5.8

Table 13: Change in pH at different temperatures

3.7.2.2. Viscosity Changes:

- **At room temperature** (25°C) and **refrigerated conditions** (4°C), no significant changes in viscosity were observed.
- **At 45°C**, formulations with higher **Triphala** content (**1:4, 1:2 ratios**) showed a decrease in viscosity, indicating some degradation or breakdown of the formulation's structure.
- **3:1** and **4:1 ratio** remained stable in terms of viscosity at all temperatures as shown in table 14.

Formulation	Initial Viscosity (Pa·s)	Viscosity at 25°C (Pa·s)	Viscosity at 4°C (Pa·s)	Viscosity at 45°C (Pa·s)
Vinca Rosea 5%	450	450	460	430
Vinca Rosea 10%	600	600	610	580
Triphala 5%	400	400	410	380

Triphala 10%	500	500	510	470
3:1 Vinca	520	520	530	510
4:1 Vinca	550	550	560	540
2:1 Vinca	530	530	540	510
1:2 Vinca	470	470	480	450
1:4 Vinca	430	430	440	410

Table 14: Changes in viscosity in different temperatures

3.7.3. Color and Texture Changes

- **Room temperature and refrigerated conditions**: No significant color or texture changes were observed in any of the formulations.
- **At 45°C**, formulations with higher **Triphala** content (**1:4, 1:2 ratios**) showed slight discoloration (darker hue) after 3 weeks, likely due to the degradation of phenolic compounds in **Triphala**.
- Formulations with higher **Vinca Rosea** content remained visually stable under all conditions.
- Formulations with higher **Triphala** content (**1:4, 1:2 ratios**) were more sensitive to elevated temperatures, showing signs of phase separation, pH fluctuations, and viscosity changes. These formulations should be stored at room temperature or refrigerated for best stability.
- Formulations with higher **Vinca Rosea** content, such as the **3:1** and **4:1 ratios**, demonstrated the best overall stability across all conditions, with minimal changes in physical and chemical properties. These formulations are more robust and suitable for various storage conditions.
- All formulations are stable at **room temperature (25°C)** and under **refrigerated conditions (4°C)**. However, elevated temperatures (45°C) may negatively affect the stability of **Triphala-rich formulations**, making them less suitable for hot climates or long-term storage in warm environments.

- This stability profile suggests that **Vinca Rosea-rich formulations** (**3:1** and **4:1**) offer the best long-term stability, making them ideal for commercial applications where storage conditions may vary.

4. Discussion:

The findings of this study underscore the significant potential of combining *Vinca Rosea* and *Triphala* for enhanced wound-healing efficacy. Phytochemical analysis confirmed the presence of key bioactive compounds, including alkaloids, tannins, and flavonoids, which are known to play pivotal roles in reducing oxidative stress and inflammation while promoting cellular repair and proliferation (Harborne, 1998). The higher yields of methanol extracts from both *Vinca Rosea* and *Triphala* suggest a predominance of polar compounds, such as phenolics and tannins, which have been extensively associated with antioxidant and wound-healing properties (Baliga et al., 2012). These findings align with studies demonstrating that phenolics can scavenge free radicals, stabilize cell membranes, and modulate inflammatory responses, thereby accelerating wound closure (Kumar et al., 2010; Mehta et al., 2018).

The formulation studies demonstrated that the combination of *Vinca Rosea* and *Triphala*, particularly at higher *Vinca Rosea* concentrations (3:1 and 4:1 ratios), provided superior occlusion and moisture retention, as indicated by a significant reduction in transepidermal water loss (TEWL). Maintaining a moist wound environment is critical for promoting keratinocyte migration and epithelialization, which are key aspects of wound healing (Bennett, Underwood, & Morrison, 2016). On the other hand, *Triphala*-rich formulations (1:4 and 1:2 ratios) exhibited better spreadability, lower viscosity, and reduced irritation potential, making them suitable for sensitive skin or frequent application scenarios.

Stability studies revealed that formulations with higher *Vinca Rosea* concentrations demonstrated better physical and chemical stability under varied storage conditions. The robustness of these formulations under elevated temperatures and humidity suggests their potential for commercialization, especially in tropical and subtropical regions where storage conditions may be challenging. Conversely, the slight instability of *Triphala*-rich formulations at elevated temperatures, evidenced by minor phase separation and pH fluctuations, emphasizes the need for

careful packaging and storage solutions to maintain their efficacy over time (Anjana et al., 2014; Panwar & Sharma, 2017).

The synergistic interplay between the bioactive compounds of *Vinca Rosea* and *Triphala* likely amplifies their wound-healing properties. *Vinca Rosea*, with its alkaloids vincristine and vinblastine, contributes to anti-inflammatory effects and collagen synthesis, while *Triphala* enhances antioxidant defenses and antimicrobial protection (Chandran & Kuttan, 2008; Nayak & Pinto Pereira, 2006). This complementary mechanism of action supports the hypothesis that combining herbal agents with distinct but overlapping therapeutic effects can lead to superior outcomes, as previously observed in studies exploring synergistic herbal formulations (Mehta et al., 2018).

Histological analyses in similar studies have shown that the use of polyherbal formulations enhances fibroblast proliferation, neovascularization, and collagen deposition compared to single-agent treatments (Prasad et al., 2019). These effects are crucial for wound contraction and tensile strength improvement. Additionally, formulations with balanced ratios of *Vinca Rosea* and *Triphala* may offer optimal results by integrating the antimicrobial and astringent properties of *Triphala* with the anti-inflammatory and pro-collagen synthesis capabilities of *Vinca Rosea*.

Future research should focus on clinical trials to validate the efficacy and safety of these formulations in human populations, particularly in chronic and diabetic wound models where oxidative stress and delayed healing are prevalent. Additionally, exploring molecular mechanisms underlying the synergistic effects of these herbs could provide valuable insights for designing targeted wound-care products. Investigating advanced delivery systems, such as hydrogels and nanoemulsions, may further enhance the bioavailability and stability of these formulations, opening new avenues for their application in wound management.

5. Conclusion

This study highlights the promising synergistic wound-healing effects of Vinca Rosea and Triphala formulations, supported by phytochemical analysis and stability assessments. The formulations, particularly those with a higher concentration of Vinca Rosea, demonstrated robust stability and efficacy in creating optimal wound-healing environments through improved occlusiveness, pH

compatibility, and bioavailability of active constituents. Stability studies further revealed that formulations rich in Vinca Rosea (3:1 and 4:1 ratios) maintained superior physical and chemical integrity under varied storage conditions, making them suitable candidates for commercialization. These findings underscore the potential of combining traditional herbal remedies to develop safe, cost-effective, and efficacious wound-care products.

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