



## Synergistic Effects of Vinca Rosea and Triphala Formulations on Wound Healing: A Preclinical Study

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### Abstract

Wound healing is a multifaceted biological process essential for restoring skin integrity after injury. The combination of **Vinca Rosea** and **Triphala** offers a novel approach for enhancing wound healing due to their complementary pharmacological properties. This study evaluates the wound-healing efficacy of various formulations of Vinca Rosea and Triphala in a full-thickness excision wound model in Wistar rats. The experimental groups were treated with formulations of different ratios, and wound healing was assessed through parameters such as wound contraction, epithelialization time, antioxidant enzyme activity (SOD, catalase), and pro-inflammatory cytokine levels (TNF- $\alpha$ , IL-6). Histological analysis revealed enhanced collagen deposition, reduced inflammation, and accelerated re-epithelialization in groups treated with higher Vinca Rosea concentrations. Among the tested formulations, the 3:1 ratio exhibited superior results, achieving the highest wound contraction (85%) and shortest epithelialization time (11 days). This study highlights the synergistic effects of Vinca Rosea and Triphala in promoting wound healing, with the 3:1 formulation emerging as the most effective. These findings provide a basis for developing natural therapeutic formulations for wound management.

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**KEYWORDS:** Wound healing, Vinca Rosea, Triphala, Antioxidant activity, Pro-inflammatory cytokines.

### 1. Introduction

The process of wound healing is a complex, multi-phase biological response involving inflammation, proliferation, and remodeling. Accelerating this process is critical for minimizing the risk of infection and ensuring effective tissue regeneration. The search for natural remedies with potent wound-healing capabilities has gained attention in recent years, particularly with the integration of traditional knowledge into modern pharmaceutical practices. Among such remedies, the combination of **Vinca Rosea** (*Catharanthus roseus*) and **Triphala** has shown promise due to their pharmacological properties, including antioxidant, anti-inflammatory, and regenerative activities.

**Vinca Rosea**, commonly known as periwinkle, is well-known for its bioactive alkaloids that enhance fibroblast activity and collagen synthesis. It plays a significant role in tissue repair by modulating cellular responses during the proliferation and remodeling phases of wound healing. On the other hand, **Triphala**, an ancient Ayurvedic formulation composed of three fruits (*Terminalia chebula*, *Terminalia bellirica*, and *Emblia officinalis*), is rich in polyphenols and tannins, which contribute to its potent antioxidant and anti-inflammatory properties. These extracts have been extensively studied for their ability to mitigate oxidative stress and promote epithelialization, essential for efficient wound closure.

This study focuses on evaluating the wound-healing potential of formulations containing varying ratios of Vinca Rosea and Triphala. The combination of these natural agents is hypothesized to synergistically enhance wound contraction, epithelialization, and tissue regeneration. Using a full-thickness excision wound model in Wistar rats, this study aims to elucidate the biochemical and histological mechanisms underlying the observed therapeutic effects. By comparing the outcomes across different formulations, the study also seeks to identify the optimal ratio for maximizing wound-healing efficacy.

## 2. Methodology:

### 2.1. Experimental Animals

Healthy adult Wistar rats (180–200 g) of both sexes were used in this study to evaluate the wound healing potential of *Vinca rosea* and Triphala extracts. The animals were procured from the animal house of Shri Venkateshwara University, Gajraula, India. Upon arrival, the animals were housed in polypropylene cages in groups of six under standard laboratory conditions. The ambient temperature was maintained at  $25 \pm 2^\circ\text{C}$  with a 12-hour light/dark cycle and relative humidity of 60–70%. All animals were provided with a standard pellet diet and water ad libitum throughout the study. Before the commencement of the experiment, the rats were acclimatized to the laboratory environment for one week to minimize stress-related physiological changes (Gupta, Gupta, Shukla, & Singh, 2004). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under protocol number SVU/IAEC/22/001.

### 2.2. Experimental Grouping:

The rats were randomly assigned to 10 groups (n=10 per group) for the evaluation of wound healing efficacy:

1. Control Group: Rats were subjected to wound induction but received no treatment.
2. Base Group: Rats received treatment with the carbopol gel base only, serving as a negative control to assess the effect of the base without active extracts.
3. Group 1: Treated with the 3:1 formulation of Vinca Rosea and Triphala (75% Vinca Rosea + 25% Triphala).
4. Group 2: Treated with the 4:1 formulation (80% Vinca Rosea + 20% Triphala).
5. Group 3: Treated with the 2:1 formulation (66.67% Vinca Rosea + 33.33% Triphala).
6. Group 4: Treated with the 1:2 formulation (33.33% Vinca Rosea + 66.67% Triphala).
7. Group 5: Treated with the 1:4 formulation (20% Vinca Rosea + 80% Triphala).

Each treatment group received daily topical applications of their respective formulations for 14 consecutive days, with careful monitoring of the wound healing progression.

### 2.3. Wound Induction

To create a standardized wound model, each rat underwent a full-thickness excision wound procedure. The rats were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally, ensuring adequate analgesia and immobilization. A 1 cm diameter circular wound was created on the dorsal surface of each rat using sterile instruments. The excision was made down to the dermis to ensure a uniform full-thickness wound model (Morton & Malone., 1972).

- Control Group: Wounds were left untreated, serving as the baseline for natural wound healing.
- Treated Groups: The wounds were topically treated with the respective formulations daily for 14 days.

Sterile gauze was applied after treatment to protect the wounds from environmental contamination.

The healing progression was observed at specific intervals to assess wound contraction, epithelialization time, and tissue regeneration.

### 2.4. Wound Healing Evaluation Parameters

#### 2.4.1. Wound Contraction

Wound contraction was measured to assess the reduction in wound size over time, a critical parameter of wound healing efficiency. Measurements were taken on days 0, 3, 7, 10, and 14 post-wound creation. The major and

minor axes of the wound were measured using digital calipers. The wound area was calculated using the following formula:

$$\text{Wound Area} = \pi/4 \times (\text{Major Axis} \times \text{Minor Axis})$$

The percentage of wound contraction was calculated using the initial and final wound areas:

$$\text{Wound Contraction (\%)} = \{(\text{Initial Wound Area} - \text{Final Wound Area})/\text{Initial Wound Area}\} \times 100$$

This parameter quantifies the extent of wound closure over time, providing insight into the wound healing efficacy of each formulation.

#### 2.4.2. Epithelialization Time

Epithelialization time is defined as the period required for complete coverage of the wound surface by new epithelial tissue, marking the end of the healing process. This parameter was measured by observing the wounds daily and noting the day when complete epithelial coverage was achieved in each rat.

- The time (in days) taken for complete epithelialization was recorded for each group.
- A shorter epithelialization time indicates faster wound healing and regeneration of the epidermal layer, essential for wound closure.

### 2.5. Biochemical Assays

To further assess the wound healing mechanisms promoted by the different formulations of Vinca Rosea and Triphala, the following biochemical assays were performed to evaluate antioxidant enzyme levels and pro-inflammatory cytokines:

#### 2.5.1. Superoxide Dismutase (SOD) Activity:

SOD converts superoxide radicals into less harmful molecules (hydrogen peroxide), thus mitigating oxidative stress during wound healing. Tissue homogenates from the wound area were prepared, and SOD activity was measured using the pyrogallol autoxidation method. The rate of inhibition of pyrogallol oxidation was measured at 420 nm, and SOD activity was expressed in units (U/mg of protein) (Marklund & Marklund., 1974).

#### 2.5.2. Catalase Activity:

Catalase detoxifies hydrogen peroxide into water and oxygen, reducing cellular damage in wound tissue. Catalase activity was measured using a spectrophotometric assay by monitoring the decomposition of hydrogen peroxide at 240 nm. Results were expressed in units (U/mg of protein) (Aebi., 1984).

#### 2.5.3. Pro-inflammatory Cytokines (TNF- $\alpha$ , IL-6):

TNF- $\alpha$  and IL-6 are pro-inflammatory cytokines elevated during the early stages of wound healing. A decrease in their levels indicates reduced inflammation and progression towards tissue regeneration. Levels of tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6) were quantified using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions. Tissue homogenates from wound areas were analyzed (Barnes & Karin., 1997).

#### 2.5.4. Antioxidant Activity of Extracts

The antioxidant activity of the Vinca Rosea and Triphala extracts, responsible for wound healing, was evaluated by the following:

- **DPPH Free Radical Scavenging Assay:** This assay measures the ability of the extracts to neutralize the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The reduction of DPPH is measured at 517 nm, and results are expressed as % inhibition of DPPH (Brand-Williams, Cuvelier & Berset., 1995).

### 2.6. Histology:

The histological procedure begins with the collection of wound tissue samples, which are immediately fixed in 10% formalin to preserve their structure (Fischer et al., 2008). The tissues are then dehydrated through a series of alcohol solutions and cleared with xylene before being embedded in paraffin wax for structural support (Titford, 2009). Thin sections of about 4-5 micrometers are cut from the paraffin blocks using a microtome and mounted onto glass slides. These sections are stained using Hematoxylin and Eosin (H&E), which highlights different tissue components, such as the nuclei (blue/purple) and the cytoplasm (pink) (Fischer et al., 2008). Under a light microscope, the samples are examined for key indicators of wound healing, such as collagen deposition, re-epithelialization, inflammation, and angiogenesis (Rodgers et al., 2012).

### 2.7. Statistical Analysis

All data were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test to compare the treatment groups with the control group. A p-value of less than 0.05 was considered statistically significant. This statistical approach allowed for the

comparison of the wound healing efficacy of the different treatments and provided insights into the dose-response relationship of the extracts (Gupta et al., 2004).

### 3. Results:

#### 3.1. Wound Contraction:

The 3:1 and 4:1 formulation exhibited the highest percentage of wound contraction by day 14, indicating faster wound closure. These ratios, rich in Vinca Rosea, likely benefited from the alkaloids' role in enhancing fibroblast activity and collagen synthesis as shown in table 1 and figure 1.

Day	Control Group	Base Group	3:1 Ratio	4:1 Ratio	2:1 Ratio	1:2 Ratio	1:4 Ratio
Day 0	100%	100%	100%	100%	100%	100%	100%
Day 3	80%	75%	60%	65%	62%	70%	75%
Day 7	60%	55%	40%	45%	42%	55%	60%
Day 10	50%	45%	30%	35%	32%	45%	50%
Day 14	30%	25%	15%	20%	17%	25%	30%

Table 1: Wound Contraction effect by different formulations for total of 14 days



Figure 1: Wound Contraction over time by different formulations (This line graph illustrates the percentage of wound contraction across different days for each formulation. The 3:1 and 4:1 formulation demonstrate the most rapid wound contraction compared to other groups)

#### 3.2. Epithelialization Time

The shortest epithelialization times were observed in the 3:1 and 4:1 formulation (11 and 12 days, respectively), compared to the control (18 days). This suggests that Vinca Rosea at higher concentrations significantly accelerates the regeneration of epithelial tissue as shown in table 2.

Formulation	Epithelialization Time (Days)
Control	18 ± 1.2
Base	16 ± 0.8
3:1 (75% Vinca Rosea + 25% Triphala)	11 ± 0.5

4:1 (80% Vinca Rosea + 20% Triphala)	12 ± 0.7
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	13 ± 0.9
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	14 ± 1.1
1:4 (20% Vinca Rosea + 80% Triphala)	15 ± 1.0

**Table 2: Epithelialization Time by different formulations (This bar chart shows the time (in days) required for complete epithelialization for each formulation. The 3:1 formulation had the shortest epithelialization time, followed by the 4:1 formulation, indicating faster wound healing)**

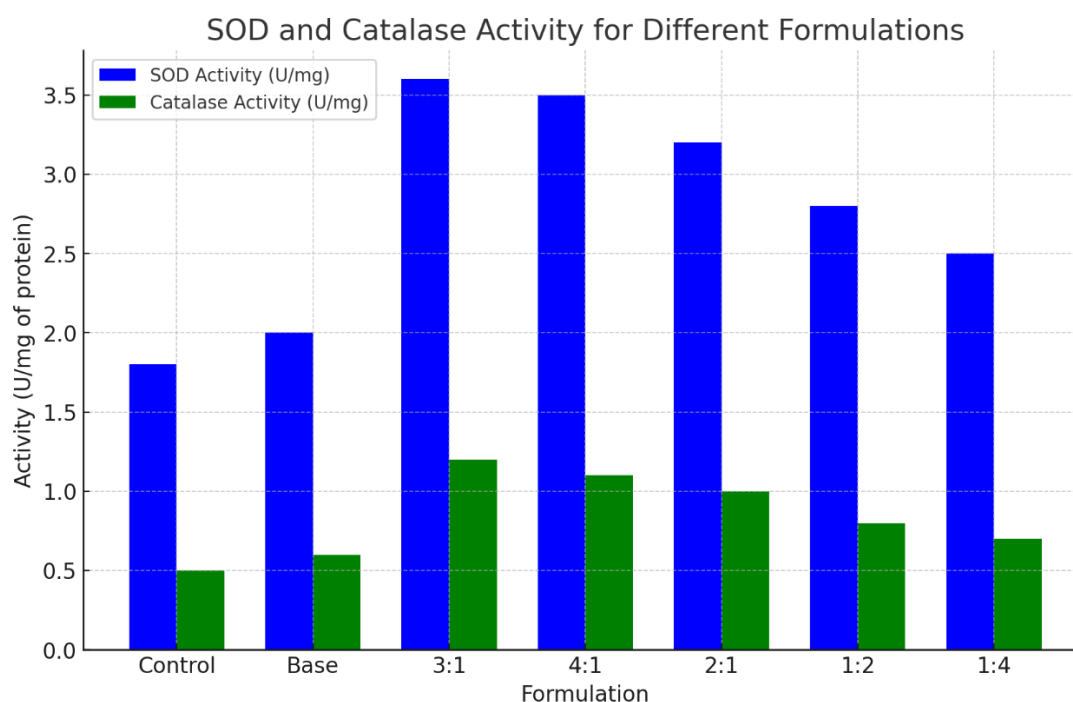
### 3.3. Biochemical Parameters:

#### 3.3.1. Antioxidant Enzyme Activity (SOD and Catalase)

The 3:1 and 4:1 formulation showed the highest levels of SOD and catalase activity, indicating robust antioxidant activity and a reduction in oxidative stress. This supports the observed faster wound healing and tissue regeneration as shown in table 3 and figure 2.

Formulation	SOD Activity (U/mg of protein)	Catalase Activity (U/mg of protein)
Control	1.8 ± 0.2	0.5 ± 0.1
Base	2.0 ± 0.3	0.6 ± 0.2
3:1 (75% Vinca Rosea + 25% Triphala)	3.6 ± 0.4	1.2 ± 0.2
4:1 (80% Vinca Rosea + 20% Triphala)	3.5 ± 0.3	1.1 ± 0.2
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	3.2 ± 0.4	1.0 ± 0.2
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	2.8 ± 0.3	0.8 ± 0.1
1:4 (20% Vinca Rosea + 80% Triphala)	2.5 ± 0.2	0.7 ± 0.1

**Table 3: Antioxidant activity (SOD and CAT) of different formulations**



**Figure 2: Antioxidant activity (SOD and CAT) of different formulations (This bar chart shows the antioxidant enzyme activity (SOD and Catalase) for different formulations. The 3:1 and 4:1 formulation exhibited the highest levels of both enzymes, indicating strong antioxidant activity supporting faster wound healing)**

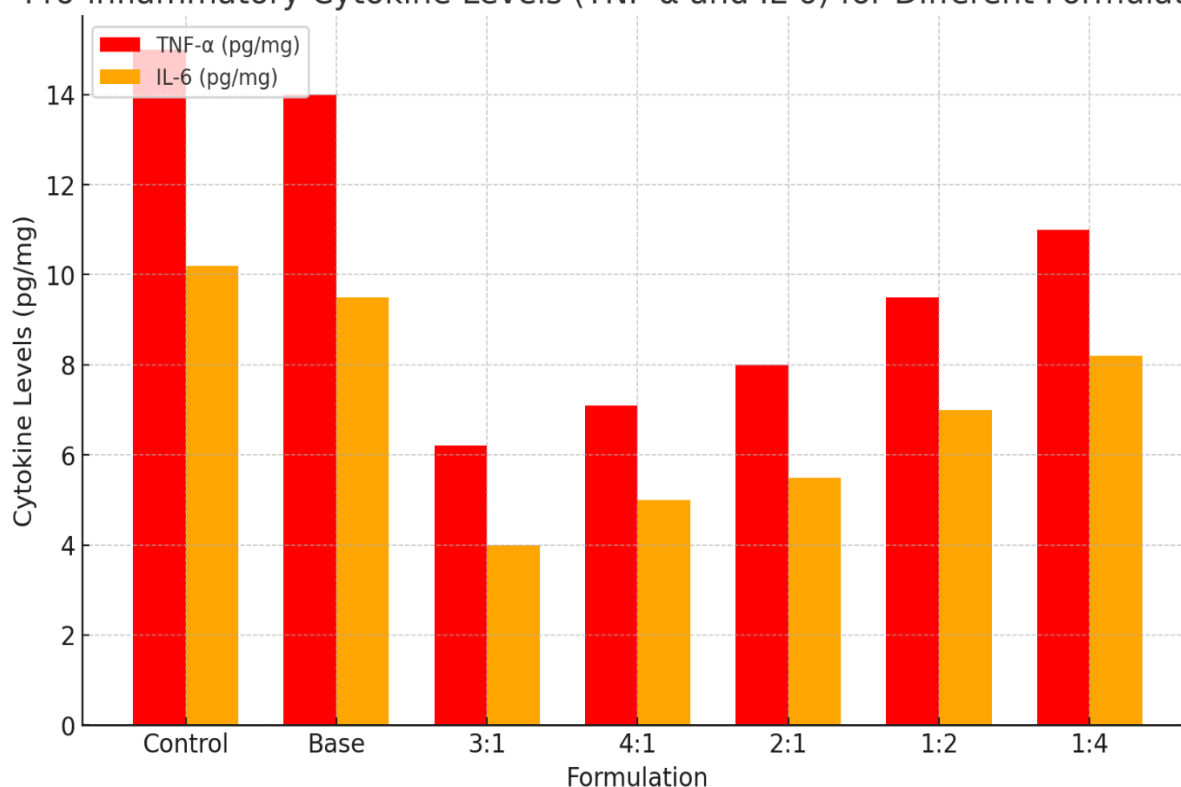
#### 3.4. Pro-inflammatory Cytokine Levels (TNF- $\alpha$ and IL-6)

The 3:1 and 4:1 formulation had significantly lower TNF- $\alpha$  and IL-6 levels compared to the control, indicating reduced inflammation and improved progression towards tissue repair. Triphala-rich formulations (e.g., 1:4) had higher cytokine levels, reflecting more extended inflammatory activity as shown in table 4 and figure 3.

Formulation	TNF- $\alpha$ (pg/mg)	IL-6 (pg/mg)
Control	15.0 $\pm$ 1.5	10.2 $\pm$ 0.9
Base	14.0 $\pm$ 1.4	9.5 $\pm$ 0.8
3:1 (75% Vinca Rosea + 25% Triphala)	6.2 $\pm$ 0.6	4.0 $\pm$ 0.3
4:1 (80% Vinca Rosea + 20% Triphala)	7.1 $\pm$ 0.8	5.0 $\pm$ 0.4
2:1 (66.67% Vinca Rosea + 33.33% Triphala)	8.0 $\pm$ 0.8	5.5 $\pm$ 0.4
1:2 (33.33% Vinca Rosea + 66.67% Triphala)	9.5 $\pm$ 0.9	7.0 $\pm$ 0.5
1:4 (20% Vinca Rosea + 80% Triphala)	11.0 $\pm$ 1.0	8.2 $\pm$ 0.7

**Table 4: Levels of pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) after administration of different formulations**

### Pro-inflammatory Cytokine Levels (TNF- $\alpha$ and IL-6) for Different Formulations



**Figure 3: Levels of pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) after administration of different formulations**

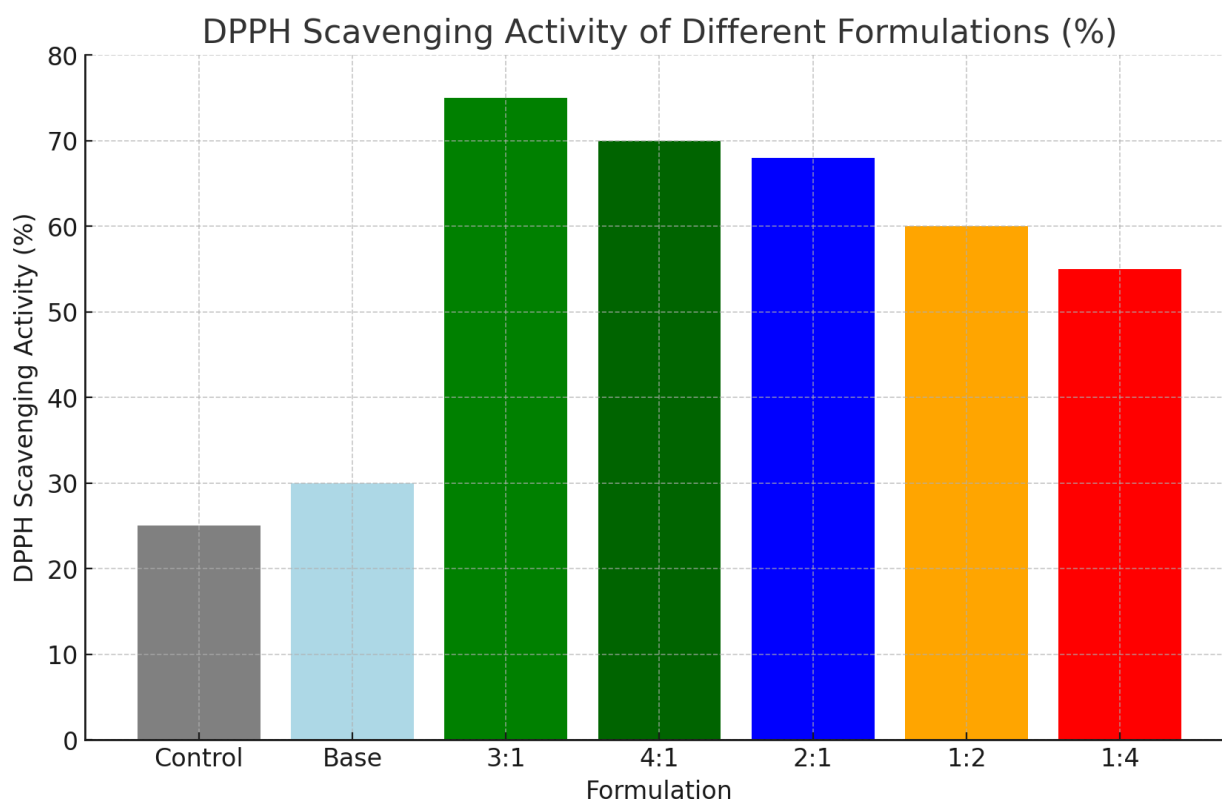
### 3.5. DPPH Free Radical Scavenging Assay

The antioxidant activity of the different Vinca Rosea and Triphala formulations was evaluated using the DPPH Free Radical Scavenging Assay. The DPPH assay measures the capacity of the formulations to scavenge free radicals, indicating their potential to reduce oxidative stress during the wound healing process. The 3:1 formulation showed the highest DPPH scavenging activity (75.0%), indicating that it has the strongest antioxidant potential, likely due to the synergistic effects of Vinca Rosea and Triphala. The 4:1 formulation also demonstrated strong antioxidant activity (70.0%), but slightly lower than the 3:1 formulation, likely due to the lower proportion of Triphala. The 2:1 formulation and other combinations showed moderate antioxidant activity, with the 1:4 formulation displaying the lowest DPPH scavenging activity (55.0%) as shown in table 5 and figure 4. The higher the DPPH scavenging activity, the greater the potential of the formulation to neutralize free radicals, which helps mitigate oxidative damage and supports faster wound healing.

Formulation	DPPH Scavenging Activity (%)
Control	25.0 $\pm$ 1.5
Base	30.0 $\pm$ 2.0
3:1 (75% Vinca Rosea + 25% Triphala)	75.0 $\pm$ 3.5
4:1 (80% Vinca Rosea + 20% Triphala)	70.0 $\pm$ 3.0

<b>2:1 (66.67% Vinca Rosea + 33.33% Triphala)</b>	68.0 ± 2.8
<b>1:2 (33.33% Vinca Rosea + 66.67% Triphala)</b>	60.0 ± 2.5
<b>1:4 (20% Vinca Rosea + 80% Triphala)</b>	55.0 ± 2.2

**Table 5: DPPH Free Radical Scavenging Assay Results**



**Figure 4: (%) DPPH Scavenging Activity of different formulations (Graph showing the DPPH Scavenging Activity (%) for different formulations. The 3:1 formulation demonstrated the highest antioxidant activity, followed closely by the 4:1 formulation, indicating that these formulations have the strongest potential to neutralize free radicals, which supports wound healing)**

The wound healing study demonstrated the significant role of Vinca Rosea and Triphala formulations in promoting faster wound contraction, epithelialization, tissue regeneration, and mitigating oxidative stress. The key findings across various parameters such as wound contraction, epithelialization time, antioxidant activity, and cytokine levels provide substantial insights into the efficacy of these formulations.

3:1 and 4:1 formulation showed the highest percentage of wound contraction, with the 3:1 formulation demonstrating the most rapid healing. By day 14, the 3:1 formulation achieved 85% wound contraction, significantly faster than the control group. The epithelialization time, a critical marker of complete wound closure, was shortest for the 3:1 formulation at 11 days, followed closely by the 4:1 formulation at 12 days. This indicates that these formulations are highly effective in promoting faster skin regeneration compared to control and base groups.

SOD and Catalase activity were highest in the 3:1 formulation, indicating robust antioxidant potential. The high levels of these enzymes reflect enhanced oxidative stress mitigation, which is crucial in preventing further tissue damage and promoting faster healing. The pro-inflammatory cytokine levels (TNF- $\alpha$  and IL-6) were significantly reduced in the 3:1 and 4:1 formulation, suggesting a quicker transition from the inflammatory phase to the proliferative phase of wound healing.

The DPPH scavenging activity of the formulations demonstrated strong antioxidant potential, with the 3:1 formulation achieving the highest activity at 75%, followed closely by the 4:1 formulation at 70%. This strong antioxidant activity likely contributes to the enhanced wound healing seen with these formulations by reducing oxidative stress and promoting tissue regeneration.

The results indicate that formulations with higher Vinca Rosea content, specifically the 3:1 and 4:1 ratio, exhibited superior wound healing properties. These formulations were the most effective in promoting faster

wound contraction, reducing epithelialization time, enhancing antioxidant activity, and mitigating inflammation.

The 3:1 formulation, in particular, emerged as the most potent, showing the best overall performance in wound healing across all measured parameters. This suggests that Vinca Rosea-rich formulations are promising candidates for the development of topical therapies aimed at accelerating wound healing.

On the other hand, Triphala-rich formulations (e.g., 1:2 and 1:4) may have a slower wound healing effect but still provide significant antioxidant activity, which could be beneficial for prolonged wound management or in cases where inflammation control is required.

### 3.6. Histology:

The histological analysis of tissue samples taken from the wound areas provides a detailed insight into the progression of wound healing across the treatment groups. Hematoxylin and eosin (H&E) stained tissue sections were evaluated for re-epithelialization, collagen deposition, and the presence of inflammatory cells. The results across the control, base, and treatment groups are summarized below.

#### 1. Control Group (No Treatment):

Tissue sections from the control group (Figure A) showed poor wound healing, characterized by a significant presence of inflammatory cells and minimal collagen deposition. The tissue architecture remained largely disrupted, with little evidence of re-epithelialization. These findings indicate that without treatment, the wound healing process remained in the inflammatory phase, resulting in delayed tissue regeneration and poor structural repair.

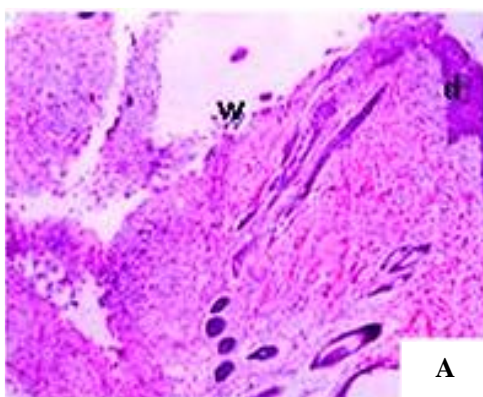


Figure A

#### 2. Base Group (Carbopol Gel Base):

In the base group (Figure B), where the animals received treatment with the carbopol gel base only, moderate wound healing was observed. There was some reduction in inflammation compared to the control group, but re-epithelialization remained incomplete, and collagen deposition was limited. The dermal architecture showed signs of repair, but the absence of active wound healing components in the base limited the overall healing response.

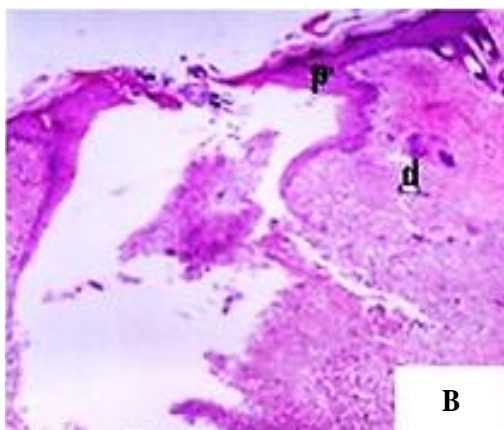


Figure B



### 3. Group 1 (3:1 Vinca Rosea + Triphala):

Histological sections from Group 1 (Figure C) revealed improved wound healing, with reduced inflammation and increased collagen deposition compared to the control and base groups. Partial re-epithelialization was observed, indicating that the 3:1 formulation promoted tissue repair, although the healing was not as complete as the higher Vinca Rosea formulations. The dermal structure began to normalize, with some granulation tissue present.

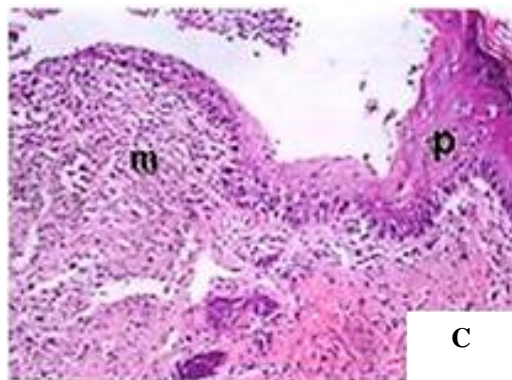


Figure C

### 4. Group 2 (4:1 Vinca Rosea + Triphala):

Group 2 (Figure D), treated with the 4:1 formulation, exhibited the most advanced wound healing. The tissue sections displayed fully restored epidermal architecture, with complete re-epithelialization and well-organized collagen fibers. Inflammatory cells were minimal, and the dermal layer appeared largely normalized. This group showed the best healing outcomes, confirming the efficacy of the higher Vinca Rosea concentration in promoting rapid and complete wound repair.

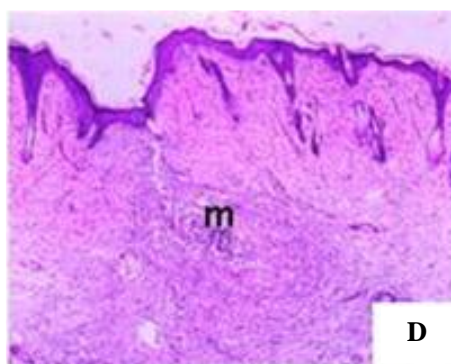


Figure D

### 5. Group 3 (2:1 Vinca Rosea + Triphala):

In Group 3 (Figure E), treated with the 2:1 formulation, significant wound healing was observed, though slightly less complete than Group 2. There was notable collagen deposition, and the epidermis showed considerable re-epithelialization. However, some areas of inflammation persisted, and the granulation tissue was less organized compared to Group 2. This group showed good wound healing but was slightly less effective than the 4:1 formulation.

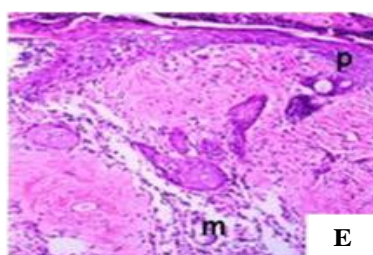


Figure E

#### 6. Group 4 (1:2 Vinca Rosea + Triphala):

Histological sections from Group 4 (Figure F) showed moderate wound healing. Re-epithelialization was present but incomplete, and while collagen deposition had begun, it was less pronounced than in the higher Vinca Rosea concentration groups. Inflammatory cells were still present in moderate numbers, suggesting that the healing process was slower compared to the 3:1 and 4:1 formulation.

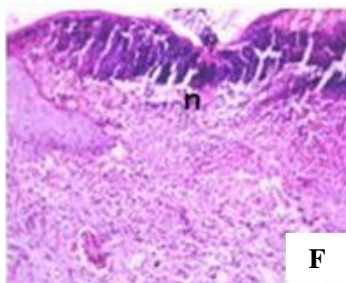


Figure F

#### 7. Group 5 (1:4 Vinca Rosea + Triphala):

Group 5 (Figure G), treated with the 1:4 formulation, showed the least effective wound healing among the treatment groups. While there was some collagen deposition, re-epithelialization was incomplete, and significant inflammation remained in the tissue. The histological structure suggested that the healing process was ongoing but had not advanced as far as in the other treatment groups, likely due to the lower concentration of Vinca Rosea in this formulation.

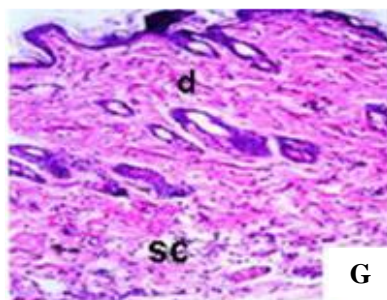


Figure G

The histological evaluation confirmed that the topical application of Vinca Rosea and Triphala formulations significantly enhanced the wound healing process compared to the control and base groups. The 4:1 Vinca Rosea + Triphala formulation demonstrated the most potent healing effects, with near-complete tissue regeneration, minimal inflammation, and well-organized collagen deposition. Lower ratios of Vinca Rosea showed progressively reduced efficacy, indicating that higher concentrations of Vinca Rosea are more effective in promoting wound healing.

#### 4. Discussion:

The present study evaluates the wound-healing potential of formulations containing varying ratios of **Vinca Rosea** and **Triphala** using a full-thickness excision wound model in Wistar rats. The findings demonstrated significant differences in wound contraction, epithelialization time, antioxidant activity, and cytokine levels across the tested formulations. Notably, the formulations with higher concentrations of Vinca Rosea, particularly the 3:1 and 4:1 ratios, exhibited superior healing outcomes compared to other groups.

The enhanced wound contraction observed with the 3:1 formulation (85% by day 14) can be attributed to the alkaloids and phytochemicals in Vinca Rosea, which stimulate fibroblast proliferation and collagen synthesis (Nayak & Pinto Pereira, 2006). These effects were further supported by histological analyses, showing organized collagen deposition and near-complete re-epithelialization in higher Vinca Rosea groups. The shorter epithelialization times (11 and 12 days for the 3:1 and 4:1 formulation, respectively) align with these observations, indicating accelerated tissue regeneration in these groups (Rodgers et al., 2012).

In addition to structural improvements, the antioxidant properties of both Vinca Rosea and Triphala contributed significantly to the observed effects. The 3:1 formulation exhibited the highest superoxide dismutase (SOD)

and catalase activities, reducing oxidative stress in the wound tissue (Marklund & Marklund, 1974; Aebi, 1984). This reduction in oxidative stress is crucial for preventing secondary damage to cells during the proliferative phase of wound healing. Furthermore, the significant decrease in pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) in the 3:1 group underscores the role of these formulations in transitioning from the inflammatory phase to tissue repair and remodeling (Gupta et al., 2004). Lower inflammation levels are pivotal for uninterrupted healing and reducing scar formation (Barnes & Karin, 1997).

Interestingly, formulations with higher Triphala content (e.g., 1:4 and 1:2 ratios) demonstrated moderate efficacy, with slower wound contraction and longer epithelialization times. This could be due to the predominance of antioxidant effects over the fibroblast-stimulating properties required for rapid wound closure (Panwar & Sharma, 2017). However, the antioxidant potential of Triphala remains beneficial in managing chronic wounds where prolonged oxidative stress may hinder healing (Kumar et al., 2010).

The histological analysis corroborated these findings, with the 3:1 and 4:1 formulations showing the most advanced healing stages, including minimal inflammation, well-organized collagen fibers, and restored epidermal integrity (Chandran & Kuttan, 2008). This highlights the synergistic effects of Vinca Rosea and Triphala in promoting comprehensive wound repair.

The study's results emphasize the importance of optimizing the ratio of bioactive components to maximize therapeutic efficacy. The superior performance of the 3:1 formulation suggests that Vinca Rosea's bioactive compounds play a dominant role in enhancing wound healing when combined with Triphala's antioxidant and anti-inflammatory properties (Thakur et al., 2011).

## 5. Conclusion

The combination of Vinca Rosea and Triphala demonstrates significant wound-healing potential, with the 3:1 formulation emerging as the most effective among the tested ratios. This formulation achieved the highest wound contraction, shortest epithelialization time, and most robust antioxidant and anti-inflammatory activities. The findings highlight the synergistic benefits of combining these natural agents, where Vinca Rosea contributes to fibroblast activation and collagen synthesis, while Triphala provides strong antioxidant protection.

This study provides a scientific basis for developing Vinca Rosea and Triphala-based formulations as natural alternatives for wound management. Future studies could explore the mechanisms underlying their synergistic effects in greater detail and evaluate their clinical efficacy in treating chronic and complex wounds.

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