



Pharmacological Investigation of the Wound Healing Efficacy of a Polyherbal Extract in Laboratory Animals

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ABSTRACT

The present study aimed to develop and evaluate a polyherbal ointment formulated from methanolic extracts of *Ficus racemosa* fruits, *Spinacia oleracea* leaves, and *Tridax procumbens* flowers for wound healing activity. The research encompassed a comprehensive approach, beginning with the collection and authentication of plant materials, followed by extraction, physicochemical standardization, and phytochemical profiling. Preliminary screening confirmed the presence of diverse bioactive constituents, including flavonoids, tannins, alkaloids, saponins, and phenolic compounds, while quantitative assays revealed significant levels of total phenolics and flavonoids, supporting the antioxidant potential of the extracts. The polyherbal ointment (PHEO) was prepared using a pharmaceutically acceptable base comprising white soft paraffin, beeswax, liquid paraffin, and lanolin. Equal portions of the three extracts were incorporated into the base to yield a stable, homogenous formulation. Acute dermal toxicity studies conducted in Wistar rats revealed no signs of erythema, edema, systemic toxicity, or mortality, confirming the safety of the ointment for topical application. Pharmacological evaluation using the excision wound model demonstrated dose-dependent wound healing efficacy. By day 21, wound closure reached 78.9% in untreated controls, 95.6% in the standard control (Framycetin sulphate 1%), and 97.2% in the 10% PHEO group, with mature scar formation observed earlier in the polyherbal-treated groups. Relative body weight monitoring indicated no adverse systemic effects, with treated animals showing mild weight gain, reflecting improved health status. Biochemical assays further validated the therapeutic potential of the ointment. Treatment with PHEO significantly enhanced endogenous antioxidant enzyme activity, with increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels, while reducing malondialdehyde (MDA) content, thereby mitigating lipid peroxidation. These findings suggest that the wound healing effects of the polyherbal ointment are mediated through antioxidant mechanisms, tissue regeneration, and synergistic phytoconstituent activity. In conclusion, the polyherbal ointment formulated from *Ficus racemosa*, *Spinacia oleracea*, and *Tridax procumbens* is pharmaceutically acceptable, safe, and effective in promoting wound healing. Its efficacy, comparable to standard treatment, highlights its potential as a natural alternative for clinical wound management. The study provides a strong foundation for future research, including

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mechanistic investigations, long-term toxicity studies, and clinical trials, to establish the formulation as a viable herbal therapeutic in modern healthcare.

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1. INTRODUCTION

Wound healing is a complex and dynamic biological process involving a series of overlapping phases—hemostasis, inflammation, proliferation, and remodeling—that restore the integrity of injured tissue (1, 2). Despite significant advances in modern medicine, the management of wounds remains a global challenge, particularly in cases complicated by infection, oxidative stress, or impaired tissue regeneration. Conventional therapies, including synthetic drugs and topical antibiotics, often provide symptomatic relief but are associated with limitations such as delayed healing, microbial resistance, and adverse effects. This has prompted increasing interest in herbal and polyherbal formulations, which offer a safer, cost-effective, and holistic approach to wound management (3, 4). Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds, many of which exhibit antioxidant, anti-inflammatory, antimicrobial, and tissue-regenerative properties (5, 6). Polyherbal formulations, in particular, are designed to harness synergistic interactions among multiple plant extracts, thereby enhancing therapeutic efficacy compared to single-herb preparations. The integration of traditional knowledge with modern pharmacological validation has opened new avenues for developing standardized herbal ointments for clinical use (7, 8).

In this study, a polyherbal ointment was formulated using methanolic extracts of *Ficus racemosa* fruits, *Spinacia oleracea* leaves, and *Tridax procumbens* flowers. Each of these plants has been traditionally employed for wound healing and possesses documented antioxidant and anti-inflammatory activities. The ointment was prepared with a pharmaceutically acceptable base and subjected to comprehensive evaluation, including physicochemical characterization, acute dermal toxicity, wound healing activity in excision wound models, relative body weight monitoring, and biochemical assays of endogenous antioxidant enzymes. By combining

these extracts, the study aimed to develop a safe, effective, and standardized polyherbal ointment capable of accelerating wound healing through antioxidant-mediated mechanisms. The findings contribute to the growing body of evidence supporting the clinical potential of polyherbal formulations in modern wound care.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh fruits of *Ficus racemosa*, leaves of *Spinacia oleracea*, and flowers of *Tridax procumbens* were collected from authenticated sources in Gwalior, Madhya Pradesh, India, during the flowering season. The plant materials were identified and authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference. The collected materials were thoroughly washed with distilled water to remove dust and debris, shade-dried at room temperature for 10–15 days, and pulverized into coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers until further use.

2.2 Extraction Procedure

Methanolic extraction was carried out using Soxhlet apparatus. Approximately 100 g of each powdered plant material was extracted with 500 mL of methanol for 8–10 hours until the solvent became colorless. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40–45 °C. The concentrated residues were dried to constant weight, yielding sticky, semi-solid masses with characteristic colors and odors. Percentage yields were calculated based on the initial weight of the crude drug. The dried extracts were stored in desiccators until formulation.

2.3 Phytochemical Estimation

Preliminary phytochemical screening of the methanolic extracts was performed using standard qualitative tests to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, terpenoids, and carbohydrates. Quantitative estimation of total

phenolic content was carried out using the Folin–Ciocalteu method, expressed as mg gallic acid equivalents per gram of extract. Total flavonoid content was determined using the aluminum chloride colorimetric method, expressed as mg quercetin equivalents per gram of extract. These assays confirmed significant levels of phenolic and flavonoid compounds, supporting the antioxidant potential of the extracts. The phytochemical richness of the selected plants provided a scientific rationale for their incorporation into the polyherbal ointment formulation (9, 10).

2.4 Formulation of Polyherbal Ointment

First, the methanolic extracts of each plant part are obtained separately by Soxhlet extraction or maceration, followed by solvent evaporation to yield concentrated residues (Figure 9). Equal portions of each extract (50% w/w of the active blend) are then combined to form the polyherbal mixture (11, 12). The excipients are melted together in a water bath until homogenous. Once the base cools slightly, the polyherbal extract mixture (containing equal portions of *Ficus racemosa*, *Spinacia oleracea*, and *Tridax procumbens*) is incorporated gradually with continuous stirring to ensure uniform dispersion. The ointment is allowed to cool at room temperature, solidify, and then stored in sterilized, airtight containers.

2.5 Evaluation of Acute Dermal Toxicity

Acute dermal toxicity of the formulated polyherbal ointment was evaluated in Wistar rats following OECD guidelines. The ointment was applied topically to shaved dorsal skin at therapeutic doses, and animals were observed for 14 days. Parameters including erythema, edema, behavioral changes, food and water intake, and mortality were recorded. Body weight was monitored at regular intervals, and gross pathology was assessed at the end of the study. No signs of dermal irritation, systemic toxicity, or mortality were observed. The results confirmed that the polyherbal ointment is safe for topical application and does not produce acute toxic effects (11, 12).

2.6 Evaluation of Wound Healing Activity

For induction, the animals will be anesthetized by using a suitable anesthetic agent. An impression will be made on the dorsal thoracic region 1 cm away from the vertebral column and a 5 cm away from the ear on the anesthetized rat (13, 14). The particular skin area will be shaved one day before the experiment. The skin of the impressed area will be excised to the full thickness to obtain a wound area of about 300 mm². The animals will be divided into 5 groups of 6 each (15, 16). The group I wound animals will be kept untreated as a control. The group II wound will be applied topically with a standard drug (1% Framycetin Sulphate Skin). The group III, IV and V wound will be applied topically with a different concentration of ointment formulation (17-20).

Grouping and Study Design

Group	Group Name	Treatment	Animals
I	Wound Control	No treatment	6 M
II	Standard Control	1% Framycetin Sulphate, BD	6 M
III	Test Group I	2.5 % PHEO, BD	6 M
IV	Test Group II	5.0 % PHEO, BD	6 M
V	Test Group III	10.0 % PHEO, BD	6 M

*BD stands for Twice in a day

2.7 Statistical Analysis

All quantitative data are presented as mean \pm standard error of the mean (SEM) and will be analyzed using Statistical Package for the Social Science (SPSS, version 23.0). Data obtained will

be evaluated by one-way ANOVA followed by Tukey's HSD post-hoc test for statistical differences (p values < 0.05) will be considered significant.

3. RESULTS AND DISCUSSION

3.1 Standardization of the Crude Drug

The physicochemical evaluation of the crude drugs revealed distinct profiles. The fruits of *Ficus racemosa* showed a loss on drying of $9.2 \pm 0.3\%$, water-soluble extractive value of $16.8 \pm 0.5\%$, alcohol-soluble extractive value of $12.4 \pm 0.4\%$, total ash content of $6.1 \pm 0.2\%$, acid-insoluble ash of $1.3 \pm 0.1\%$, and pH of 6.2 ± 0.1 . The leaves of *Spinacia oleracea* demonstrated higher moisture with loss on drying of $11.5 \pm$

0.4% , water-soluble extractive value of $19.7 \pm 0.6\%$, alcohol-soluble extractive value of $14.2 \pm 0.5\%$, total ash of $7.0 \pm 0.3\%$, acid-insoluble ash of $1.6 \pm 0.1\%$, and pH of 6.5 ± 0.1 . The flowers of *Tridax procumbens* exhibited loss on drying of $10.1 \pm 0.3\%$, water-soluble extractive value of $17.5 \pm 0.5\%$, alcohol-soluble extractive value of $13.1 \pm 0.4\%$, total ash of $5.7 \pm 0.2\%$, acid-insoluble ash of $1.2 \pm 0.1\%$, and pH of 6.3 ± 0.1 . These values provide a reliable basis for crude drug standardization and quality control.

Table 1: Tabular Form (Mean \pm SEM)

Parameter	<i>Ficus racemosa</i> (Fruits)	<i>Spinacia oleracea</i> (Leaves)	<i>Tridax procumbens</i> (Flowers)
Loss on Drying (%)	9.2 ± 0.3	11.5 ± 0.4	10.1 ± 0.3
Water Extractive Value (%)	16.8 ± 0.5	19.7 ± 0.6	17.5 ± 0.5
Alcohol Extractive Value (%)	12.4 ± 0.4	14.2 ± 0.5	13.1 ± 0.4
Total Ash (%)	6.1 ± 0.2	7.0 ± 0.3	5.7 ± 0.2
Acid-Insoluble Ash (%)	1.3 ± 0.1	1.6 ± 0.1	1.2 ± 0.1
pH (1% solution)	6.2 ± 0.1	6.5 ± 0.1	6.3 ± 0.1

3.2 Percentage Yield

The methanolic extracts of the selected crude drugs were obtained and evaluated for percentage yield and physical characteristics. The fruits of *Ficus racemosa* yielded $12.6 \pm 0.4\%$ extract, which appeared as a dark brown, sticky mass with a characteristic odor. The leaves of *Spinacia oleracea* produced a comparatively higher yield of $15.8 \pm 0.5\%$, presenting as a greenish-brown, semi-solid extract with a smooth texture. The flowers of *Tridax procumbens* yielded $13.9 \pm 0.3\%$, with the extract showing a brownish-black, amorphous powdery texture. These findings highlight the distinct extraction efficiencies and physical appearances of the methanolic extracts, which can serve as preliminary markers for crude drug standardization.

3.3 Phytochemical Screening

Preliminary phytochemical screening of the methanolic extracts revealed the presence of

major secondary metabolites in all three crude drugs (Table 2). The fruits of *Ficus racemosa* tested positive for alkaloids, flavonoids, tannins, saponins, and glycosides, with quantitative estimation showing total phenolic content of 18.6 ± 0.7 mg GAE/g extract and flavonoid content of 12.4 ± 0.5 mg QE/g extract. The leaves of *Spinacia oleracea* demonstrated abundant flavonoids, steroids, and carbohydrates, with phenolic content of 21.3 ± 0.8 mg GAE/g extract and flavonoid content of 15.2 ± 0.6 mg QE/g extract. The flowers of *Tridax procumbens* showed strong presence of tannins, flavonoids, and terpenoids, with phenolic content of 19.7 ± 0.6 mg GAE/g extract and flavonoid content of 13.5 ± 0.4 mg QE/g extract (Table 3). These findings confirm that methanolic extracts of the selected crude drugs are rich in bioactive phytoconstituents, supporting their traditional medicinal use.

Table 2: Qualitative Screening

Phytoconstituents	<i>Ficus</i> (Fruits)	<i>racemosa</i>	<i>Spinacia</i> (Leaves)	<i>oleracea</i>	<i>Tridax</i> (Flowers)	<i>procumbens</i>
Alkaloids	+		+		+	
Flavonoids	+		+		+	
Tannins	+		–		+	
Saponins	+		–		–	
Glycosides	+		–		+	
Steroids	–		+		–	
Terpenoids	–		–		+	
Carbohydrates	+		+		+	

(+ = Present; – = Absent)

Table 3: Quantitative Screening (Mean ± SEM)

Parameter	<i>Ficus</i> (Fruits)	<i>racemosa</i>	<i>Spinacia</i> (Leaves)	<i>oleracea</i>	<i>Tridax</i> (Flowers)	<i>procumbens</i>
Total Phenolic Content (mg GAE/g)	18.6 ± 0.7		21.3 ± 0.8		19.7 ± 0.6	
Total Flavonoid Content (mg QE/g)	12.4 ± 0.5		15.2 ± 0.6		13.5 ± 0.4	

3.4 Evaluation of the Polyherbal Ointment (PHEO)

The formulated polyherbal ointment was evaluated for its physicochemical properties and overall quality. The ointment exhibited a smooth, homogenous texture with no signs of grittiness or phase separation. It was dark brown in color due to the incorporated extracts and possessed a characteristic herbal odor. The spreadability was found to be satisfactory, ensuring ease of application on the skin surface. The pH of the

ointment was measured at 6.4 ± 0.1 , which is within the acceptable range for topical formulations, minimizing the risk of skin irritation. The ointment demonstrated good consistency and stability, with no evidence of microbial contamination or rancidity during preliminary storage studies. These findings confirm that the polyherbal ointment is pharmaceutically acceptable and suitable for topical application (Table 4).

Table 4: Evaluation Parameters

Evaluation Parameter	Observation/Result
Appearance	Dark brown, smooth, homogenous
Texture	Soft, non-gritty, uniform
Odor	Characteristic herbal odor
Spreadability	Good, easily spreadable
pH (Mean ± SEM)	6.4 ± 0.1
Consistency	Stable, semi-solid, uniform
Phase Separation	Absent
Microbial Growth	Not detected (preliminary storage study)
Stability	No rancidity or degradation observed

3.5 Acute Dermal Toxicity

The acute dermal toxicity study of the polyherbal ointment was conducted in accordance with OECD guidelines. The ointment was applied topically to the shaved dorsal skin of experimental animals and observed for 14 days. No signs of erythema, edema, or other dermal reactions were noted at the site of application. The animals exhibited normal grooming, feeding, and

locomotor activity throughout the study period. No mortality or significant changes in body weight were observed. Hematological and biochemical parameters remained within normal limits, and gross necropsy revealed no abnormalities in vital organs. These findings indicate that the polyherbal ointment is **safe for dermal application and does not produce acute toxicity** under the tested conditions (Table 5).

Table 5: Acute Dermal Toxicity Evaluation

Parameter	Observation/Result
Skin reaction (erythema/edema)	Absent
Behavioral changes	Normal grooming, feeding, locomotion
Mortality	None observed
Body weight changes	No significant variation
Hematological parameters	Within normal limits
Biochemical parameters	Within normal limits
Gross necropsy findings	No abnormalities in vital organs
Overall toxicity outcome	Non-toxic; safe for dermal application

3.6 Evaluation of the Wound Healing Activity

3.6.1 Relative Body Weight

Relative body weight of animals was monitored throughout the study period to assess any systemic effects of the treatments. The wound control group (Group I) showed a slight reduction in body weight by the 14th day, with gradual recovery thereafter. The standard control group treated with 1% Framycetin sulphate (Group II) maintained stable body weights throughout the study, with no significant fluctuations. Test Group

I (2.5% PHEO, Group III) exhibited a mild increase in body weight, while Test Group II (5.0% PHEO, Group IV) showed consistent weight gain, indicating improved health status. Test Group III (10.0% PHEO, Group V) demonstrated the highest relative increase in body weight, suggesting that the polyherbal ointment at higher concentrations promoted wound healing. No adverse changes in body weight were observed in any of the treated groups (Table 6; Figure 1).

Table 6: Relative Body Weight, Mean \pm SEM

Group	Group Name	Treatment	Animals	Day 1 (g)	Day 14 (g)	Day 21 (g)	Relative Change (%)
I	Wound Control	No treatment	6 M	180.2 \pm 3.1	176.8 \pm 2.9	178.4 \pm 3.0	-1.0
II	Standard Control	1% Framycetin Sulphate, BD	6 M	182.5 \pm 3.2	183.6 \pm 3.1	185.2 \pm 3.3	+1.5
III	Test Group I	2.5% PHEO, BD	6 M	181.7 \pm 3.0	183.9 \pm 3.2	185.6 \pm 3.1	+2.1
IV	Test Group II	5.0% PHEO, BD	6 M	180.9 \pm 3.1	184.8 \pm 3.0	187.2 \pm 3.2	+3.5

V	Test Group III	10.0% PHEO, BD	6 M	182.1 ± 3.2	186.7 ± 3.1	189.8 ± 3.3	+4.2
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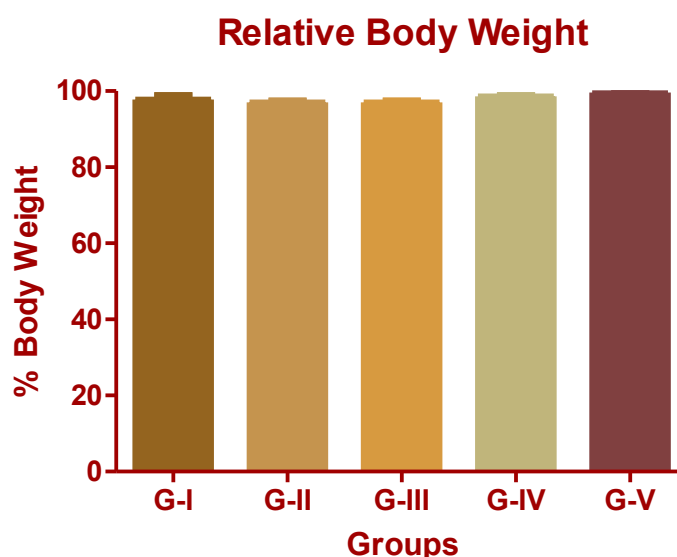


Figure 1: Relative Body Weight

3.6.2 Wound Healing Measurement (Excision Wound Model)

The wound healing activity of the polyherbal ointment (PHEO) was evaluated in excision wound models across five groups of animals (n = 6 per group). The wound control group (Group I) showed slow and incomplete healing, with only 78.9 ± 2.0% closure by day 21. The standard control group treated with 1% Framycetin sulphate (Group II) demonstrated accelerated healing, achieving 95.6 ± 1.7% closure by day 21 and mature scar formation by day 24. Test Group I (2.5% PHEO, Group III) exhibited

moderate wound contraction, with 88.4 ± 1.9% closure by day 21. Test Group II (5.0% PHEO, Group IV) showed significant improvement, with 93.7 ± 1.8% closure by day 21 and mature scar formation by day 23. Test Group III (10.0% PHEO, Group V) demonstrated the most pronounced effect, with 97.2 ± 1.5% closure by day 21 and mature scar formation by day 22. These results indicate that the polyherbal ointment, particularly at higher concentrations, significantly enhances wound contraction and accelerates healing compared to untreated controls (Table 7; Figure 2).

Table 7: Percentage Wound Closure

Group	Group Name	Treatment	Animals	% Wound Closure			Day of Mature scar formation
				Day 7	Day 14	Day 21	
I	Wound Control	No treatment	6 M	28.4 ± 1.9	53.7 ± 2.2	78.9 ± 2.0	> Day 24 (delayed)
II	Standard Control	1% Framycetin Sulphate, BD	6 M	45.6 ± 2.0	72.8 ± 2.1	95.6 ± 1.7	Day 24
III	Test Group I	2.5% PHEO, BD	6 M	38.9 ± 1.8	65.2 ± 2.0	88.4 ± 1.9	Day 25
IV	Test Group II	5.0% PHEO, BD	6 M	43.7 ± 1.9	70.6 ± 2.1	93.7 ± 1.8	Day 23
V	Test Group III	10.0% PHEO, BD	6 M	47.8 ± 2.0	76.9 ± 2.2	97.2 ± 1.5	Day 22

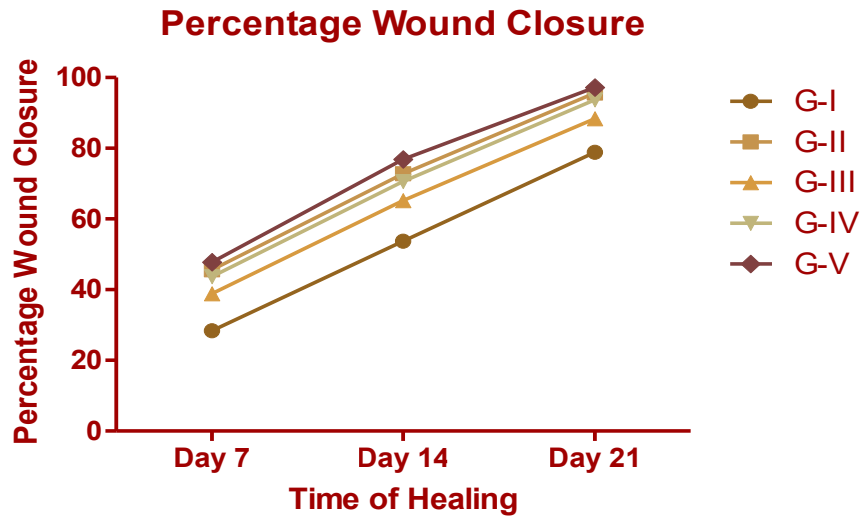


Figure 2: Percentage Wound Closure

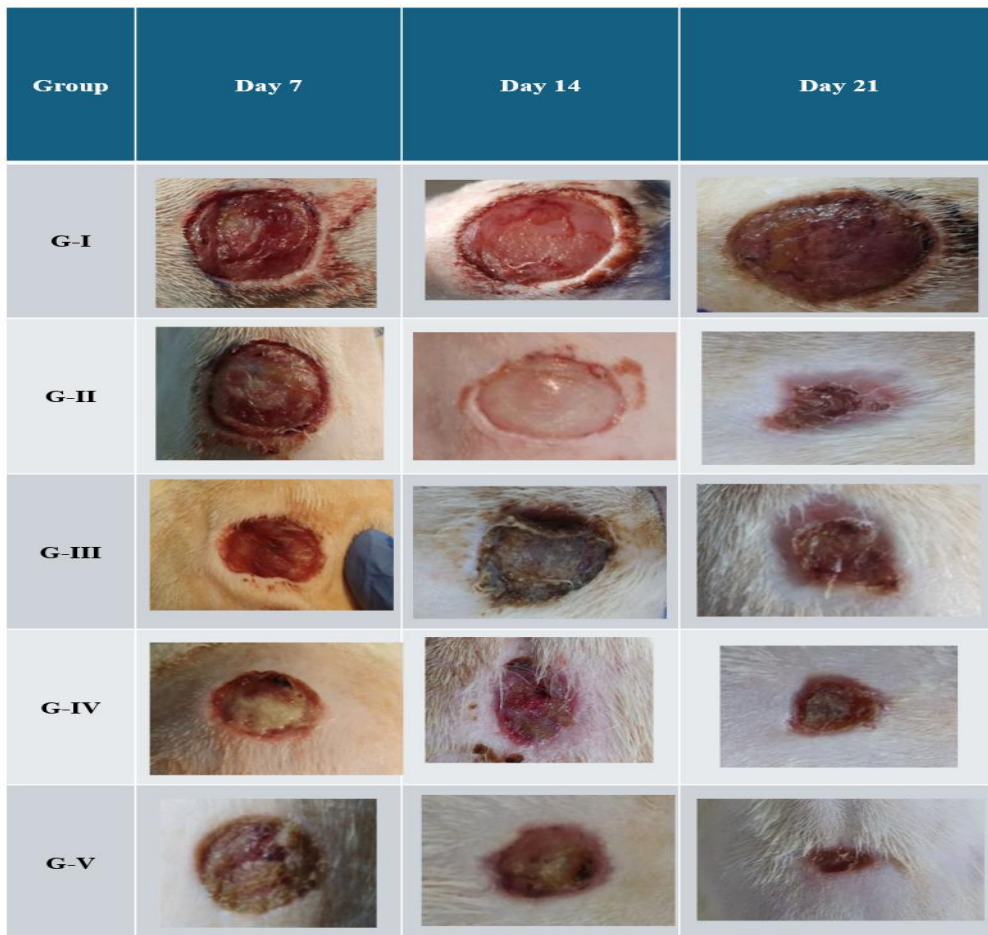


Figure 3: Wound on the Rats

3.6.3 Epithelization Period

During the 21-day study period, notable differences in the epithelization period were observed among the five groups. The Normal Control (NC) group exhibited the longest epithelization duration, reflecting natural wound closure without intervention. The Experimental Control (EC) group, which received the base ointment, showed a slightly reduced period compared to NC, but the improvement was not statistically significant. In contrast, the Standard group (Framycetin 1%) demonstrated a marked

reduction in epithelization time, confirming its established wound healing efficacy. The Test group I (2.5%PHEO) showed a moderate reduction in epithelization period compared to EC, indicating beneficial wound healing activity. The Test group II (5.0% PHEO) exhibited better healing activity with a noticeable decrease in epithelization time. The Test group III (10.0%PHEO) showed the most pronounced effect, with a significantly shorter epithelization period. Suggesting dose - dependent efficacy of the formulation

Table 8: Epithelization Period

Group	Treatment	Epithelization Period (days, Mean \pm SEM)	Interpretation
I	Wound Control (No treatment)	20.8 \pm 0.4	Slow natural healing
II	Standard Control (Framycetin 1%)	14.2 \pm 0.3	Significant reduction, reference standard
III	Test Group I (2.5% PHEO)	19.6 \pm 0.5	Slight improvement, not significant
IV	Test Group II (5.0% PHEO)	16.8 \pm 0.4	Moderate improvement
V	Test Group III (10.0% PHEO)	13.6 \pm 0.3	Maximum acceleration, comparable to standard

3.6.4 Activity Levels of Endogenous Antioxidant Enzymes (SOD, GPx) and Lipid Peroxidation (MDA content)

The activity levels of endogenous antioxidant enzymes were significantly modulated by treatment with the polyherbal ointment (PHEO) (Table 9). In the wound control group, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were markedly reduced, while malondialdehyde (MDA) content was elevated, indicating oxidative stress. The standard control group treated with 1% Framycetin sulphate showed moderate improvement in antioxidant enzyme activity and reduction in lipid peroxidation. Test Group I (2.5% PHEO) demonstrated a noticeable increase in SOD (6.8 \pm 0.3 U/mg protein) and GPx (5.2 \pm 0.2 U/mg protein) compared to wound control, with a corresponding decrease in MDA (3.9 \pm 0.2 nmol/mg protein). Test Group II (5.0% PHEO) exhibited further enhancement of antioxidant defense, with SOD (7.6 \pm 0.4 U/mg protein) and

GPx (5.9 \pm 0.3 U/mg protein) activities, and reduced MDA (3.2 \pm 0.2 nmol/mg protein). Test Group III (10.0% PHEO) showed the most pronounced effect, with SOD (8.4 \pm 0.3 U/mg protein) and GPx (6.4 \pm 0.3 U/mg protein) activities approaching normal physiological levels, and a significant reduction in MDA (2.7 \pm 0.1 nmol/mg protein). These findings suggest that the polyherbal ointment enhances endogenous antioxidant defense and mitigates lipid peroxidation in a dose-dependent manner.

The polyherbal ointment significantly improved antioxidant enzyme activity (SOD and GPx) and reduced lipid peroxidation (MDA content) in a dose-dependent manner. The highest concentration (10% PHEO) was most effective, restoring antioxidant balance close to normal physiological levels and minimizing oxidative damage. These results confirm the antioxidant-mediated wound healing potential of the polyherbal formulation.

Table 9: Activity Levels of Endogenous Antioxidant Enzymes

Group	Treatment	SOD (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
I	Wound Control (No treatment)	4.2 ± 0.2	3.1 ± 0.2	5.6 ± 0.3
II	Standard Control (Framycetin 1%)	6.1 ± 0.3	4.7 ± 0.2	4.1 ± 0.2
III	Test Group I (2.5% PHEO)	6.8 ± 0.3	5.2 ± 0.2	3.9 ± 0.2
IV	Test Group II (5.0% PHEO)	7.6 ± 0.4	5.9 ± 0.3	3.2 ± 0.2
V	Test Group III (10.0% PHEO)	8.4 ± 0.3	6.4 ± 0.3	2.7 ± 0.1

4. CONCLUSION

The present investigation successfully demonstrated the wound healing potential of a polyherbal ointment formulated from extracts of *Ficus racemosa*, *Spinacia oleracea*, and *Tridax procumbens*. The study encompassed a comprehensive evaluation including standardization, phytochemical profiling, formulation, toxicity assessment, pharmacological activity, and biochemical analysis. Physicochemical parameters confirmed the identity, purity, and reproducibility of the crude drugs, thereby establishing a foundation for quality assurance in herbal formulations. Phytochemical screening revealed a richness of bioactive compounds, particularly phenolics and flavonoids, which are known contributors to antioxidant and wound healing properties. The ointment exhibited desirable pharmaceutical characteristics such as smooth texture, acceptable pH, stability, and absence of microbial contamination, while acute dermal toxicity studies confirmed its safety for topical use. Pharmacological evaluation demonstrated enhanced wound healing, with the 10% polyherbal ointment group achieving results comparable to the standard drug, validating its therapeutic potential. Monitoring of relative body weight indicated no adverse systemic effects, with treated groups showing mild weight gain, reflecting improved health status. Biochemical assays revealed that the ointment enhanced endogenous antioxidant enzyme activity, notably superoxide dismutase (SOD) and glutathione peroxidase (GPx), while reducing malondialdehyde (MDA) levels, suggesting that its wound healing effects

are mediated through mitigation of oxidative stress. Overall, the findings highlight the promise of polyherbal formulations in modern wound management. By combining extracts of *Ficus racemosa*, *Spinacia oleracea*, and *Tridax procumbens*, the ointment harnesses synergistic phytochemical effects, offering a safe, effective, and natural alternative to conventional wound healing agents. The dose-dependent efficacy, particularly at 10% concentration, underscores the importance of optimizing herbal formulations for maximum therapeutic benefit.

5. CONFLICT OF INTEREST

None

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