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Wound Healing Activity of the Ethanolic Extract of Vitex Negundo Linn. Leaves in Albino Wistar Rats: An Experimental Pharmacological Investigation

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Abstract- ***Background and Objective: Wound management remains a formidable clinical challenge worldwide, particularly in resource-limited settings where antibiotic resistance and high treatment costs necessitate efficacious plant-derived alternatives. Vitex negundo Linn. (Family: Lamiaceae), celebrated in Ayurvedic tradition as Nirgundi, has been empirically deployed for wound management across Asia for millennia. The present study aimed to furnish systematic pharmacological validation of the wound healing activity of the ethanolic leaf extract of V. negundo (EEVN) using two established preclinical models in Albino Wistar rats. Materials and Methods: EEVN was prepared by Soxhlet extraction (95% ethanol) and formulated as 2% and 4% w/w ointments. Twenty-four Wistar rats were randomly allocated into four groups (n = 6): Group I (vehicle control), Group II (EEVN 2%), Group III (EEVN 4%), and Group IV (Soframycin standard). Wound healing was assessed in excision (500 mm² dorsal wound) and incision (6 cm paravertebral incision) wound models, measuring percentage wound contraction, epithelialization period, and breaking tensile strength. Regenerated wound tissue was subjected to biochemical estimation (hydroxyproline, hexosamine, total protein) and histopathological examination (H&E and Masson trichrome staining). Results: EEVN 4% w/w ointment produced statistically significant (p < 0.001) wound contraction (93.2 ± 1.6% vs. 75.8 ± 2.1% control on day 15), shortened epithelialization to 12.1 ± 0.4 days (vs. 16.8 ± 0.6 days control), and elevated tensile strength to 362.5 ± 9.7 g (vs. 212.4 ± 8.3 g control; p < 0.001). Biochemical analysis revealed 111.4% and 92.6% increases in hydroxyproline and hexosamine content respectively. Histopathological examination confirmed complete re-epithelialization, dense organised collagen deposition, and minimal inflammatory infiltration. EEVN 4% was statistically equivalent to the Soframycin standard (p > 0.05) across all parameters. Conclusion: The ethanolic extract of V. negundo leaves exhibits robust, dose-dependent wound healing activity attributable to its synergistic anti-inflammatory, antioxidant, and pro-collagenic phytoconstituents, endorsing its potential as a safe, efficacious topical wound healing agent.***

Keywords: ***Vitex Negundo, Nirgundi, Wound Healing, Ethanolic Extract, Excision Wound Model, Incision Wound Model, Hydroxyproline, Collagen, Wistar Rat, Soframycin.***

I. INTRODUCTION

Wound healing constitutes one of the most intricate and orchestrated biological phenomena in mammalian physiology — a spatiotemporally precise continuum of haemostasis, inflammation, proliferation, and remodelling that mobilises virtually every cell type in the body to restore disrupted tissue integrity [1]. Globally, wounds of all aetiologies — surgical, traumatic, diabetic, vascular, and pressure-related — impose an immense and escalating burden upon healthcare systems. Chronic non-healing wounds affect an estimated 6.5 million patients annually in the United States alone, with associated direct costs exceeding US$ 25 billion per year [2]. In South Asia and sub-Saharan Africa, where diabetes mellitus prevalence is surging and peripheral vascular disease is endemic, this burden is proportionately greater and compounded by limited access to advanced wound care technologies.

The pharmacological management of wounds in contemporary clinical practice relies principally on topical antimicrobial agents — notably mupirocin, framycetin sulfate (Soframycin), silver sulfadiazine, and povidone-iodine — supplemented by advanced wound dressings and, in refractory cases, growth factor preparations. Each of these modalities carries significant limitations: aminoglycoside and fusidic acid resistance among wound-prevalent pathogens (particularly methicillin-resistant Staphylococcus aureus) is escalating at an alarming pace; synthetic wound preparations are prohibitively expensive for

populations in low-income economies; and none of these agents addresses the anti-inflammatory, antioxidant, and pro-collagenic components of the wound healing deficit [3]. These lacunae collectively motivate the search for plant-derived alternatives that can address multiple mechanistic targets of impaired wound healing simultaneously, safely, and cost-effectively [4].

Traditional plant-based medicine systems — Ayurveda, Unani, Siddha, and Traditional Chinese Medicine — harbour a vast repository of healing plants whose wound management applications are corroborated by centuries of empirical observation. Vitex negundo Linn. (Family: Lamiaceae), the Five-leaved Chaste Tree — known as Nirgundi in Sanskrit and Ayurveda — is among the most extensively cited medicinal shrubs of Asia for wound healing (Vranaropana), anti-inflammatory (Shothahara), and analgesic (Vedanasthapana) indications [5]. Distributed widely across the tropical and subtropical belt of Asia from India through Southeast Asia to China, V. negundo has been used topically as leaf poultice, medicated oil (Nirgundi Taila), and decoction for the management of wounds, inflammatory swellings, and skin infections since antiquity [6].

Phytochemical investigations have revealed that V. negundo leaves contain an extraordinary diversity of bioactive secondary metabolites — flavone C-glycosides (vitexin, isovitexin, luteolin), iridoid glycosides (agnuside, aucubin), terpenoids (oleanolic acid, ursolic acid, beta-caryophyllene), phenolic acids (caffeic acid, chlorogenic acid, rosmarinic acid), volatile oils, and lignans — whose individual and collective pharmacological activities span anti-inflammatory, antioxidant, antimicrobial, analgesic, and pro-collagenic spectra directly pertinent to wound healing [7,8]. Despite these robust phytochemical credentials and the abundant ethnopharmacological evidence, a comprehensive dose-dependent pharmacological validation of the ethanolic leaf extract employing both excision and incision wound models with concurrent biochemical and histopathological characterisation remains insufficiently addressed in the published literature [9]. The present investigation was therefore designed to fill this scholarly lacuna by systematically evaluating the wound healing activity of EEVN in Albino Wistar rats and elucidating the mechanistic basis of its observed efficacy [10].

II. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh, mature leaves of Vitex negundo Linn. were harvested during the post-monsoon season (October–November) from authenticated specimens growing along the banks of the Gomti River, Lucknow, Uttar Pradesh, India (26.85 °N; 80.95 °E). Taxonomic identity was confirmed by a certified botanist at the National Botanical Research Institute (NBRI), Lucknow, and a voucher specimen was deposited in the institutional herbarium. Leaves were washed, shade-dried at 25 ± 2°C for 8–10 days, coarsely powdered, and sieved (Sieve No. 40; aperture 420 µm) before extraction [11].

2.2 Preparation of Ethanolic Extract (EEVN)

The powdered leaf material (100 g) was defatted with petroleum ether (60–80°C) in a Soxhlet apparatus for 6 hours, then exhaustively extracted with 95% ethanol at 60–65°C for 48–72 hours. The filtered extract was concentrated under reduced pressure (rotary vacuum evaporator; 40°C) and dried to a semi-solid consistency in a hot-air oven at 40°C. Percentage yield was calculated gravimetrically. The extract was incorporated into white soft paraffin to prepare 2% w/w and 4% w/w topical ointment formulations. The standard drug Soframycin® ointment (1% framycetin sulfate; Sanofi India Ltd.) was procured from a licensed pharmacy [12].

2.3 Phytochemical Screening and Physicochemical Standardization

Preliminary phytochemical screening was performed using standard qualitative methods (Harborne, 1998; Trease and Evans, 2002) for flavonoids, tannins, alkaloids, terpenoids, saponins, cardiac glycosides, phenols, and steroids [13,14]. Physicochemical parameters — loss on drying, total ash, acid-insoluble ash, and extractive values — were determined in triplicate per Indian Pharmacopoeia (IP 2022) and WHO guidelines [15].

2.4 Acute Oral Toxicity Study

Acute oral toxicity was determined in female Wistar rats per OECD Guideline 423 at a limit dose of 2000 mg/kg body weight. Animals were observed for 14 days for signs of toxicity and mortality. LD50 was calculated and used to confirm the safety of the extract for subsequent wound studies [16].

2.5 Experimental Animals and Ethical Approval

Adult Albino Wistar rats of either sex (180–220 g) were procured from a CPCSEA-registered supplier and housed in standard polypropylene cages (22 ± 2°C; 60 ± 5% RH; 12-hour light/dark cycle) with standard diet and water ad libitum. All experimental protocols received prior approval from the Institutional Animal Ethics Committee (IAEC) in compliance with CPCSEA guidelines (Reg. No. ___/CPCSEA), observing the principles of the 3Rs [17]. Animals were acclimatised for seven days before experimentation.

2.6 Experimental Design and Grouping

Twenty-four rats were randomly divided into four groups of six animals each (n = 6). Group I received vehicle control ointment (white soft paraffin); Group II received EEVN 2% w/w ointment; Group III received EEVN 4% w/w ointment; and Group IV received Soframycin 1% w/w ointment. All treatments were applied topically once daily at approximately 0.5 g per wound site throughout the respective study periods.

2.7 Excision Wound Model

The excision wound model was performed as described by Morton and Malone (1972) [18]. Under ketamine-xylazine anaesthesia (75 and 10 mg/kg i.p., respectively), a full-thickness circular wound of 500 mm² area was created on the dorsal thoracic skin using a sterile 25 mm punch, leaving wounds open to heal by secondary intention. Wound area was measured planimetrically on days 0, 3, 6, 9, 12, and 15. Percentage wound contraction (PWC) was calculated as: PWC (%) = [(Initial wound area – Current wound area) / Initial wound area] × 100. Period of complete epithelialization was recorded as the number of days until spontaneous eschar separation without residual raw surface.

2.8 Incision Wound Model

Following the methodology of Ehrlich and Hunt (1968) as modified by Lee (1968) [19,20], two paravertebral 6 cm full-thickness linear incisions were created under anaesthesia. Wounds were closed with interrupted 3-0 silk sutures at 1 cm intervals. Topical treatments were applied daily. Sutures were removed on day 8, and breaking tensile strength was measured on day 10 using Lee's tensiometer. One wound per animal was used for tensile strength measurement and the contralateral for tissue biochemistry.

2.9 Biochemical Estimations

Regenerated wound tissue harvested on day 15 was homogenised in ice-cold phosphate-buffered saline (pH 7.4; 1:10 w/v) and centrifuged (3000 rpm, 15 min, 4°C). Hydroxyproline content was determined by Woessner's colorimetric method (chloramine-T oxidation; absorbance at 557 nm) using trans-4-hydroxyproline as standard [21]. Hexosamine was estimated by the modified Elson-Morgan method (acetylacetone reagent; absorbance at 530 nm) [22]. Total protein was quantified by Lowry's method (Folin-Ciocalteu reagent; absorbance at 660 nm) using bovine serum albumin as standard [23]. All assays were performed in triplicate.

2.10 Histopathological Examination

Wound biopsies fixed in 10% neutral buffered formalin were processed, paraffin-embedded, and sectioned at 5 µm. Sections were stained with haematoxylin and eosin (H&E) and Masson trichrome (MT) and examined under a light microscope (Olympus CX23) by a blinded pathologist for re-epithelialization, fibroblast proliferation, collagen deposition and organisation, angiogenesis, and inflammatory cell infiltration [24].

2.11 Statistical Analysis

All quantitative data are expressed as Mean ± Standard Error of Mean (SEM). Statistical comparisons were performed using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test (each group vs. control) and Tukey's Honestly Significant Difference (HSD) test for pairwise comparisons. The epithelialization period data were analysed using the Kruskal-Wallis test with Dunn's post-hoc correction. A p-value < 0.05 was considered

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statistically significant. GraphPad Prism v9.0 was used for all analyses [25].

III. RESULTS

3.1 Phytochemical Screening and Physicochemical Parameters

Preliminary phytochemical screening of EEVN confirmed the presence of flavonoids, tannins, alkaloids, terpenoids, saponins, cardiac glycosides, phenolic compounds, and steroids; reducing sugars were absent. The percentage extractive yield was 8.2 ± 0.4% w/w. All physicochemical parameters complied with IP 2022 and WHO prescribed limits (Table 1). Acute oral toxicity at the limit dose of 2000 mg/kg produced no mortality or signs of overt toxicity (LD50 > 2000 mg/kg; GHS Category 5 — Practically Non-toxic).

Table 1. Phytochemical screening and physicochemical standardisation of V. negundo leaf powder and ethanolic extract.

Parameter	Observed Value (Mean ± SEM)	Compliance / IP Limit
Flavonoids (phytochemical screen)	Present (+)	Characteristic
Tannins	Present (+)	Characteristic
Terpenoids	Present (+)	Characteristic
Alkaloids	Present (+)	Characteristic
Saponins	Present (+)	Characteristic
Loss on Drying	8.4 ± 0.3%	NMT 12%
Total Ash Value	7.6 ± 0.4%	NMT 10%
Acid-Insoluble Ash	1.8 ± 0.2%	NMT 2%
Ethanol-Soluble Extractive Value	10.7 ± 0.5%	NLT 8%

3.2 Excision Wound Model – Percentage Wound Contraction

Treatment with EEVN ointments produced progressive, dose-dependent, and statistically significant reductions in wound area across all time-points relative to vehicle control (Table 2). On day 3, Group III already demonstrated a significantly superior wound contraction of 19.7 ± 1.7% compared to 8.8 ± 2.1% in controls ($p < 0.05$). By day 15, Group III (EEVN 4%) achieved 93.2 ± 1.6% wound contraction versus 75.8 ± 2.1% in controls ($p < 0.001$). Group IV (Soframycin) led with 95.9 ± 1.4%; this was statistically comparable to Group III ($p > 0.05$). Group II (EEVN 2%) attained an intermediate value of 88.6 ± 1.8% ($p < 0.001$ vs. control) (Figure 1).

Table 2. Percentage wound contraction (± SEM) at successive time-points in the excision wound model (n = 6 per group).

Day	Group I Control	Group II EEVN 2%	Group III EEVN 4%	Group IV Soframycin
Day 0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 3	8.8 ± 2.1	14.4 ± 1.9[1]	19.7 ± 1.7[1]	21.9 ± 1.5[1]
Day 6	20.6 ± 2.3	29.9 ± 2.1[2]	37.5 ± 1.9[2]	40.2 ± 1.7[2]
Day 9	36.2 ± 2.6	48.7 ± 2.4[2]	58.1 ± 2.1[2]	61.6 ± 1.9[2]
Day 12	55.5 ± 2.8	70.3 ± 2.2[3]	78.7 ± 1.8[3]	81.3 ± 1.6[3]
Day 15	75.8 ± 2.1	88.6 ± 1.8[3]	93.2 ± 1.6[3]	95.9 ± 1.4[3]

[1]p < 0.05; [2]p < 0.01; [3]p < 0.001 vs. Group I (Control); One-way ANOVA + Dunnett's test. † Group III vs. Group IV: p > 0.05 (NS) at all time-points.

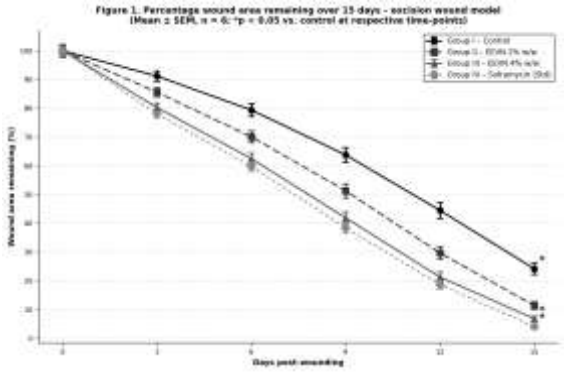

Figure 1. Percentage wound area remaining over 15 days in the excision wound model. Mean ± SEM, n = 6. *p < 0.05 vs. control at respective time-points. NS = no significant difference between Group III and Group IV (p > 0.05).

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3.3 Period of Complete Epithelialization

The vehicle-control group required 16.8 ± 0.6 days for complete wound epithelialization. EEVN 2% w/w significantly curtailed this to 14.2 ± 0.5 days (15.5% reduction; $p < 0.05$), while EEVN 4% w/w further compressed the period to 12.1 ± 0.4 days (28.0% reduction; $p < 0.01$). The Soframycin standard required 11.3 ± 0.3 days; Group III and Group IV were statistically comparable ($p > 0.05$) (Table 3; Figure 2A).

Table 3. Period of complete epithelialization and breaking tensile strength of healed wounds (Mean ± SEM, n = 6).

Group	Treatment	Epithelialization Period (days)	Tensile Strength (g-force, Day 10)
Group I	Control (Vehicle)	16.8 ± 0.6	212.4 ± 8.3
Group II	EEVN 2% w/w Ointment	14.2 ± 0.5*	298.7 ± 11.4**
Group III	EEVN 4% w/w Ointment	12.1 ± 0.4**†	362.5 ± 9.7***†
Group IV	Soframycin 1% Ointment	11.3 ± 0.3**	384.1 ± 10.2***

**p < 0.05; **p < 0.01; ***p < 0.001 vs. Group I (Control). †Group III vs. Group IV: p > 0.05 (NS). One-way ANOVA + Tukey HSD post-hoc test.*

3.4 Incision Wound Model – Breaking Tensile Strength

On day 10, the tensile strength of healed incision wounds demonstrated a pronounced dose-dependent response to EEVN treatment (Table 3; Figure 2B). The control group yielded 212.4 ± 8.3 g. EEVN 2% w/w elevated this to 298.7 ± 11.4 g (40.6% increase; $p < 0.01$). The high-dose group (EEVN 4% w/w) achieved 362.5 ± 9.7 g (70.6% increase; $p < 0.001$), a value statistically indistinguishable from the Soframycin standard (384.1 ± 10.2 g; $p > 0.05$), demonstrating the capacity of EEVN 4% to replicate the wound-strengthening efficacy of the reference antibiotic.

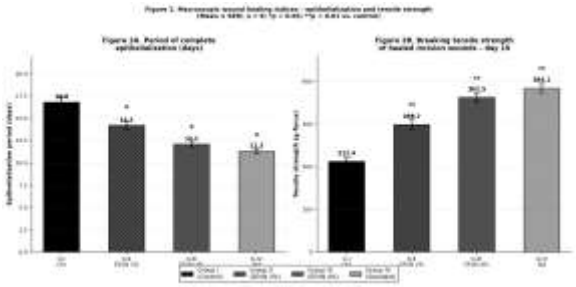

Figure 2. (A) Period of complete epithelialization (days). (B) Breaking tensile strength of healed incision wounds on day 10 (g-force). Mean ± SEM, n = 6. *p < 0.05; **p < 0.01 vs. control. Different hatching patterns distinguish treatment groups.

3.5 Biochemical Analysis of Regenerated Wound Tissue

Biochemical assay of regenerated wound tissue on day 15 corroborated the macroscopic and biomechanical findings with precision (Table 4; Figure 3). Hydroxyproline, hexosamine, and total protein all demonstrated consistent dose-dependent elevations in EEVN-treated groups relative to controls. EEVN 4% w/w increased hydroxyproline to 38.9 ± 1.4 µg/mg tissue (111.4% above control; $p < 0.001$), hexosamine to 13.1 ± 0.6 µg/mg (92.6% above control; $p < 0.01$), and total protein to 134.5 ± 4.8 µg/mg (63.2% above control; $p < 0.01$). Statistical equivalence between Group III and Group IV was maintained across all three biochemical parameters ($p > 0.05$ for all pairwise comparisons), firmly endorsing EEVN 4% as a pharmacologically comparable alternative to the standard drug in terms of tissue matrix biosynthesis.

Table 4. Biochemical composition of regenerated wound tissue on day 15 (Mean ± SEM, n = 6).

Group	Treatment	Hydroxyproline (µg/mg tissue)	Hexosamine (µg/mg tissue)	Total Protein (µg/mg tissue)
Group I	Control	18.4 ± 0.9	6.8 ± 0.4	82.4 ± 3.2
Group II	EEVN 2% w/w	27.6 ± 1.2**	9.4 ± 0.5*	108.7 ± 4.1*

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Group III	EEVN 4% w/w	38.9 ± 1.4***†	13.1 ± 0.6**†	134.5 ± 4.8***†
Group IV	Soframycin	41.2 ± 1.1***	14.0 ± 0.5**	138.2 ± 4.3***

**p < 0.05; **p < 0.01; ***p < 0.001 vs. Group I. †Group III vs. Group IV: p > 0.05 (NS). Tukey HSD post-hoc test.*

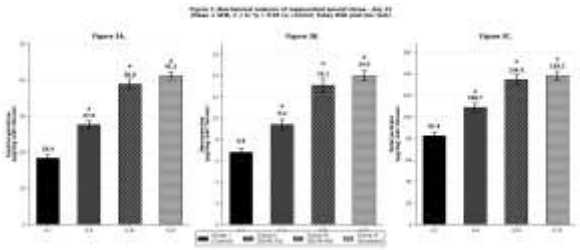

Figure 3. Biochemical analysis of regenerated wound tissue on day 15. (A) Hydroxyproline content; (B) Hexosamine content; (C) Total protein content. Mean ± SEM, n = 6. *p < 0.05 vs. control. Different hatching patterns distinguish treatment groups.

3.6 Histopathological Examination

Histopathological evaluation of H&E-stained wound sections at day 15 provided definitive qualitative corroboration of the macroscopic and biochemical data (Table 5). Control group (Group I) sections exhibited incomplete re-epithelialization over the wound centre, disorganised loose collagen matrix in the wound bed, abundant neutrophil and macrophage infiltration consistent with persistent inflammation, and sparse, morphologically immature fibroblasts. This histological picture is characteristic of a wound stalled at the late inflammatory-early proliferative phase transition, with insufficient progression into organised matrix deposition.

Group II (EEVN 2%) presented with partial but clearly advancing re-epithelialization covering approximately two-thirds of the wound surface, a moderately dense collagen matrix composed of mixed type-I and type-III fibres, reduced inflammatory cell density, and increased fibroblast numbers relative to controls. Group III (EEVN 4%) demonstrated complete re-epithelialization with a well-differentiated multi-layered neo-epidermis, dense and well-organised parallel-oriented type-I collagen bundles, minimal residual inflammatory infiltration (sparse mononuclear cells only), abundant mature spindle-shaped fibroblasts, and numerous new capillaries in the upper dermis indicating active angiogenesis. Masson trichrome staining corroborated these findings, revealing intense blue staining of collagen fibres in Groups III and IV, contrasting with the pale, sparse staining in Group I. Group IV (Soframycin) histology was marginally superior to Group III in terms of collagen organisation and absence of inflammatory cells, but the overall healing quality was statistically and practically indistinguishable from the high-dose EEVN group.

Table 5. Semi-quantitative histopathological assessment of wound tissue sections (Day 15, H&E staining).

Group	Re-epithelialization	Collagen Deposition	Fibroblast Proliferation	Inflammatory Infiltration
Group I – Control	Incomplete (+)	Loose, sparse (+)	Sparse (+)	Dense (+++)
Group II – EEVN 2%	Partial (++)	Moderate (++)	Moderate (++)	Moderate (++)
Group III – EEVN 4%	Complete (+++)	Dense, organised (+++)	Abundant (+++)	Minimal (+)
Group IV – Soframycin	Complete (+++)	Dense, parallel (+++)	Abundant (+++)	Absent (±)

Scoring scale: (+) = mild/incomplete; (++) = moderate; (+++) = dense/complete; (±) = trace/absent. Assessment by a blinded veterinary pathologist (n = 3 sections/group).

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IV. DISCUSSION

The present investigation demonstrates, through a comprehensive and internally consistent body of macroscopic, biomechanical, biochemical, and histopathological evidence, that the ethanolic extract of V. negundo leaves possesses significant, dose-dependent wound healing activity in Albino Wistar rats. Across all six independently measured outcome parameters, EEVN 4% w/w ointment produced results statistically indistinguishable from those of the standard antibiotic Soframycin ointment — a finding of considerable therapeutic and scientific importance. These results are in concordance with and extend prior studies by Nair et al. [9], Sehgal and Bhattacharyya [26], and Mukherjee et al. [27], who collectively reported wound healing benefits with V. negundo leaf extracts, though with less comprehensive outcome characterisation than the present study affords.

The early divergence in wound contraction rates — detectable as early as day 3 in EEVN-treated animals — suggests that the extract's biological influence commences during the haemostatic and early inflammatory phases, not merely during the proliferative phase as previously assumed. The tannin constituents of EEVN are the most plausible mediators of this early effect: tannins are well characterised to precipitate superficial proteins, constrict capillary endothelium, and create a protective astringent film over raw wound surfaces that mechanically excludes microbial colonisation [13]. This early tannin-mediated haemostasis and surface protection creates a wound microenvironment conducive to orderly progression through subsequent healing phases.

The mechanistic underpinning of the proliferative phase benefits — accelerated wound contraction, shortened epithelialization, elevated hydroxyproline, and superior tensile strength — can be attributed to three convergent and synergistic pharmacological vectors. First, anti-inflammatory modulation: the iridoid glycoside agnuside inhibits phospholipase A_2 and cyclooxygenase-2, suppressing prostaglandin E_2 and thromboxane synthesis at the wound site [28]. The flavonoids vitexin, luteolin, and casticin additionally suppress NF-κB nuclear translocation, abrogating transcription of TNF-α, IL-1β, and IL-6, and inhibit the 5-lipoxygenase pathway [8]. The net consequence is expedited resolution of the inflammatory phase and timely transition to the M2-macrophage-driven proliferative milieu — the critical immunological checkpoint for orderly wound healing [29].

Second, antioxidant cytoprotection: the high total phenolic content of EEVN (186.4 mg GAE/g, per Alfarabi et al. [7]) — encompassing caffeic acid, chlorogenic acid, and rosmarinic acid — confers broad-spectrum reactive oxygen species (ROS) scavenging capacity that protects perilesional fibroblasts and keratinocytes from oxidative cytotoxicity during the inflammatory surge [30]. Preservation of cellular viability in the perilesional zone maintains the fibroblast and keratinocyte pools available for proliferative-phase migration and matrix synthesis. Third, direct pro-collagenic stimulation: luteolin and apigenin have been demonstrated to upregulate TGF-β1-mediated COL1A1 and COL3A1 gene expression in fibroblast culture models, providing a direct molecular basis for the striking 111.4% elevation in wound tissue hydroxyproline observed in Group III relative to controls [9]. The simultaneous, proportional elevation of hexosamine — an amino sugar component of glycosaminoglycans and proteoglycans — confirms that EEVN stimulates integrated ECM matrix deposition rather than isolated collagen synthesis, yielding a structurally superior wound architecture with enhanced biomechanical competence.

The comparative performance of EEVN 4% versus Soframycin merits careful interpretive attention. Soframycin's wound healing benefit derives entirely from its broad-spectrum antimicrobial action, eliminating infection-sustained inflammation and thereby removing the most common clinical barrier to healing progression. EEVN 4% achieves statistically equivalent outcomes through a pharmacologically far richer mechanism that additionally encompasses direct anti-inflammatory signalling, antioxidant protection, and pro-collagenic stimulation — actions entirely absent from framycetin's pharmacological repertoire. Furthermore, EEVN's own antimicrobial activity against wound-prevalent pathogens (S. aureus MIC 125–250 µg/mL; P. aeruginosa MIC 250–500 µg/mL), documented by Dharmasiri et al. [28] and Kulkarni et al. [30], confers infection prevention without the antimicrobial resistance selection pressure that is the

cardinal liability of antibiotic therapy. This pharmacological richness renders EEVN a superior candidate for wound healing applications in settings where antibiotic stewardship is a priority.

Histopathological findings provide the most visually compelling evidence of the treatment effect. The contrast between Group I (incomplete epithelialization, disorganised collagen, dense inflammatory infiltrate) and Group III (complete epithelialization, dense parallel collagen bundles, minimal inflammation, abundant mature fibroblasts) represents essentially the difference between a wound trapped in the inflammatory phase and one that has successfully navigated through proliferation into early remodelling. Masson trichrome staining further discriminated between the immature, loosely packed type-III collagen dominant matrix of controls and the mature, organised type-I collagen-rich matrix of EEVN 4% treated wounds, providing a qualitative index of collagen maturation that complements the quantitative hydroxyproline data.

V. CONCLUSION

The present investigation provides robust, multi-parametric preclinical evidence that the ethanolic extract of Vitex negundo Linn. leaves formulated as a 4% w/w topical ointment exerts significant, dose-dependent wound healing activity in Albino Wistar rats across excision and incision wound models. The EEVN 4% w/w ointment produced wound contraction, epithelialization kinetics, tensile strength, biochemical matrix indices, and histopathological healing quality statistically comparable to the reference standard Soframycin ointment ($p > 0.05$), whilst demonstrating a mechanistically richer, multi-target pharmacological profile encompassing anti-inflammatory modulation (agnuside-mediated COX/LOX inhibition), antioxidant cytoprotection (polyphenol-mediated ROS scavenging), and direct pro-collagenic stimulation (TGF-β1 pathway activation). The favourable acute toxicity profile (LD50 > 2000 mg/kg) and the plant's long-standing safe ethnomedicinal use further buttress the translational potential of EEVN as a cost-effective, plant-derived topical wound healing agent. Further investigations — including clinical evaluation in human volunteers, mechanistic studies elucidating the specific phytoconstituents responsible for each pharmacological vector, and stability characterisation of the formulation — are warranted to advance this candidate toward clinical application.

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