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PRELIMINARY EVALUATION OF THE WOUND-HEALING ACTIVITY OF NOVINA ENNE, A POLYHERBAL OIL, USING INCISION AND EXCISION WOUND MODELS IN WISTAR RATS

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ABSTRACT
Wound healing is a complex biological process, and polyherbal oils are traditionally used for wound management. This study evaluated the wound-healing activity of Novina Enne, a traditional Ayurvedic polyherbal oil, in incision and excision wound models in Wistar rats. Animals were divided into four groups (n = 6 per group): Control, Standard (Aloe vera gel), Test I (gingelly oil), and Test II (Novina Enne). Wound-breaking strength was assessed in the incision model, while wound contraction and epithelialization period were evaluated in the excision model. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered significant. Novina Enne group showed significantly greater wound-breaking strength than the control, standard, and gingelly oil (Test I) groups ($p < 0.0001$, $p < 0.0001$, and $p < 0.001$, respectively). Wound contraction was also significantly greater in the Novina Enne group (Test II) than in the comparator groups on days 4 and 16. All treatment groups showed significantly shorter epithelialization periods than the control group ($p < 0.0001$), with no significant differences among the treatment groups. These preliminary findings suggest that Novina Enne has wound-healing potential in experimental rat models. Further studies are needed to establish its mechanisms of action, active constituents, and safety.

KEYWORDS: Novina Enne, polyherbal oil; wound healing; wound contraction; wound-breaking strength; period of epithelisation

INTRODUCTION
Wound healing is a complex and dynamic biological process that involves the coordinated interplay of cellular, humoral, and molecular mechanisms (Reinke and Sorg, 2012). The wound-healing process involves four overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling (Sim et al., 2022). Disruption of any of these phases may impair the normal wound-healing process and lead to delayed or abnormal tissue repair (Cullen et al., 2002). Poor wound healing may be caused by factors such as ageing, malnutrition, infection, poor blood supply, smoking, underlying diseases, and certain medications. These factors can delay wound repair and lead to chronic wounds, such as diabetic ulcers, vascular ulcers, and pressure ulcers (Demidova-Rice et al., 2012).
People in rural parts of India traditionally use locally prepared herbal oils for wound care. Many medicinal plants contain phytochemicals with documented antimicrobial, anti-inflammatory, analgesic, and antioxidant properties that may contribute to tissue repair and wound healing (Shobha et al., 2025, Shobha et al., 2024, Laxminarayana et al.,2011) Therefore, considerable research is being conducted to identify plant-derived bioactive compounds that can promote wound healing.
In ayurveda practice, oils are used as one of the modes of application in treatment of the wounds (Lahorkar et al., 2009). The present study was undertaken to evaluate the wound-healing potential of Novina Enne, a traditional polyherbal oil manufactured by Shri Nithyananda Aushadhalaya, Mangalore, using incision and excision wound models in Wistar rats (Laxminarayana et al.,2011). Traditionally, herbal oil preparations use gingelly oil (sesame oil), derived from Sesamum indicum, is commonly used as a carrier oil. It contains fatty acids and other bioactive constituents that may and it is mentioned to influence skin hydration and tissue repair. In the present study Novina Enne, a traditional poly herbal preparation is used to study its wound healing effect where gingelly oil is used as base in this preparation.
As the wound-healing effects of the formulation cannot be fully evaluated through in vitro studies or directly in humans, animal models are used to investigate its efficacy and safety under controlled experimental conditions. Although Novina Enne is marketed as a pain-relief oil, it is reportedly widely used by local communities for wound care and the management of bedsores, in addition to relieving pain. However, scientific evidence supporting its wound-healing efficacy remains limited or unavailable traditionally
We hypothesize that topical application of Novina Enne influences wound healing in Wistar rats, as assessed by wound-breaking strength, percentage wound contraction, and the period of epithelialization, compared with an untreated control, Aloe vera gel, and gingelly oil. As gingelly oil itself may affect wound healing, its inclusion as a separate treatment group helps assess the contribution of the base oil relative to the complete polyherbal formulation. The study aimed to generate preliminary experimental evidence regarding the wound-healing potential of this traditional formulation

Aim and Objectives

To evaluate the wound-healing activity of Novina Enne using incision and excision wound models in Wistar rats.

- To evaluate the effect of Novina Enne on wound-breaking strength using the incision wound model.
- To assess wound contraction rate (percentage wound contraction) and period of epithelialization following topical application of Novina Enne using the excision wound model.

MATERIALS AND METHODS:

Experimental animals:

Healthy, inbred Wistar rats of either sex, weighing 200–250 g, were used in the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). The animals were housed in a CPCSEA-registered animal facility under standard environmental conditions, with a temperature of 23 ± 2°C, relative humidity of 50 ± 5%, and a 10–14-hour light–dark cycle. Standard rat feed and water were provided ad libitum. Following wound induction, the animals were housed individually in polypropylene cages containing husk bedding until the end of the experimental period.

Experimental study groups

Four groups of Wistar rats were used, n=6 rats /group

- Group I: Control- No application- To observe wound healing without topical treatment
- Group II: Standard- Aloe vera gel- To compare healing with a reference topical preparation
- Group III: Test1-Gingelly oil- To assess the contribution of the base oil
- Group IV: Test II- Novina Enne [Poly herbal oil]- To evaluate the wound-healing potential of the complete formulation

Drugs used: Injection ketamine [50mg/kgb.w, (Laxminarayana et al.,2011)] procured from drug house, Aloe vera gel [Patahnjali]and Gingelly oil [Tila taila] purchased from local distributer, Novina Enne ;Poly Herbal oil- prepared from Shri Nithyananda Oshadhalaya – Mangalore, India .Composition of Novina Enne oil is : each 10 ml oil contains 0.3mg pastes from each herbs such as -Syzygium Aromaticum [flower bud], Nigella Sativa seeds, roots of Rubia cordifolia , rhizome of zingiber officinale, Aloe barbadensis , resin raktabola (Commiphora myrrha) , Acasia Catechu bark, Ferula asafoetida, cinnamoneum comphora (camphor), in gingelly oil 10 ml . Ready prepared oil available from local stores was used in this study

Wound Induction

Incision wound model

The animals were fasted for 12 hours before the experiment. Anaesthesia was induced by intraperitoneal administration of ketamine at a dose of 50 mg/kg body weight (Laxminarayana et al.,2011). After shaving the dorsal region, two straight, paravertebral incisions, each measuring 6 cm in length, were made through the full thickness of the skin using a sterile, sharp blade, with one incision on either side of the vertebral column. After the wound edges were gently dried, the incisions were closed using 4-0 silk sutures with a straight, round-bodied needle. Interrupted sutures were placed at equidistant intervals of 1 cm. The wounds were then cleaned with cotton swabs soaked in 70% alcohol. Following wound induction, the animals were housed individually (Laxminarayana et al.,2011).

Excision wound model

After fasting for 12 hours, the rats were anaesthetized by intraperitoneal administration of ketamine at a dose of 50 mg/kg body weight. The dorsal thoracic region was shaved, and a circular area measuring 2.5 cm in diameter was marked on the skin, approximately 5 cm from the ears. The full-thickness skin within the marked area was excised to create a wound with an approximate area of 500 mm² (Morton and Malone, 1972, Almeida et al., 2017). Hemostasis was achieved by gently mopping the wound with cotton swabs soaked in warm saline. Following wound induction, the animals were housed individually in separate cages.

Drug application on wounds

Four groups of animals (n = 6 per group) were used for each of the incision and excision wound models. All treatments were administered topically. Group I served as the control group, in which the wounds were cleaned using sterile gauze moistened with sterile water. Group II served as the standard treatment group and received topical Aloe vera gel, with two drops applied to each incision wound and one drop to each excision wound. Group III received gingelly oil, with three drops applied to each incision wound and three drops to each excision wound. Group IV received Novina Enne herbal oil, with three drops applied to each incision wound and \

drops to each excision wound. The treatments were administered twice daily, as specified in the treatment protocol. The day of wound induction was designated as day 0. In the incision wound model, the treatments were administered from day 0 to day 9 post-wounding. In the excision wound model, the animals were treated from day 1 to day 21 post-wounding. (Laxminarayana et al.,2011).

Parameters assessed

Incision wound model: The sutures were removed on the 7th post-wounding day. On the 10th post-wounding day, wound-breaking strength was measured using the continuous, constant water-flow technique. Wound-breaking strength was expressed as the minimum weight of water [in grams] required to produce gaping of the wound (Brown et al., 1988).

Excision wound model: Two physical attributes of healing namely wound contraction rate and epithelisation period were studied. To monitor wound contraction the progressive changes in wound area will be followed planimetrically. Wound

area were traced on a transparent paper on day 0, 4, 8,12 and 16. The tracings were then transferred to 1mm graph sheets and the wound area was measured. Percentage of wound contraction was calculated using the following equation:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size (500 mm2)} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelisation period: Period of epithelisation was monitored by noting the number of days required for the eschar to fall off leaving no raw wound behind (Laxminarayana et al.,2011).

Statistical analysis

Data was analyzed using SPSS 16. The results were expressed as mean ± SD. Differences between the experimental groups were compared using one-way ANOVA followed by Tukey's multiple-comparisons test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Wound breaking strength

In incision wound model, Wound-breaking strength was compared among the control, standard (Aloe vera gel), gingelly oil, and Novina Enne groups using one-way ANOVA followed by Tukey's multiple-comparisons test. Wound-breaking strength was significantly higher in the standard, gingelly oil (Test I), and Novina Enne (Test II) groups than in the control group ($p < 0.0001$). No statistically significant difference was observed between the standard and gingelly oil groups ($p = 0.1373$). However, the Novina Enne (Test II) group exhibited significantly greater wound-breaking strength than both the standard group ($p < 0.0001$) and the gingelly oil (Test I) group. These findings indicate that Novina Enne treatment was associated with greater wound-breaking strength than the other groups under the conditions of this experiment($p<0.001$), [Figure 1].

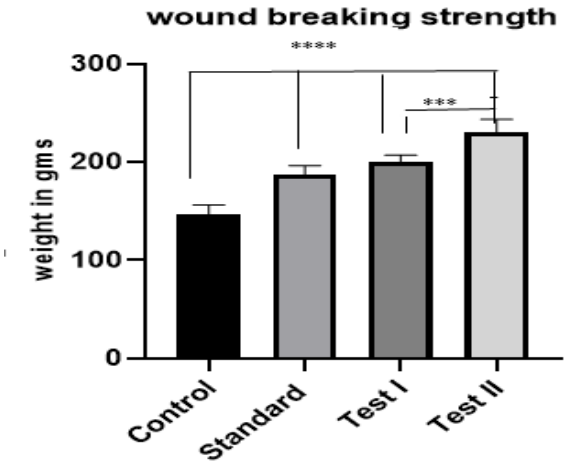


Figure 1: Effect of Novina Enne (Test II) on wound-breaking strength in the incision wound model in Wistar rats. Wound-breaking strength was expressed as the minimum weight of water (gms) required to produce wound gaping. Data were analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test. *$p < 0.001$ was level of significance. Control vs standard, control vs gingelly oil (Test I) and control vs Novina Enne (Test II) ****$p < 0.0001$; Test I vs Test II, $p< 0.001$.

Wound contraction rate (percentage wound contraction)

On day 4, The percentage of wound contraction was compared among the control, standard (Aloe vera gel), gingelly oil (Test I), and Novina Enne (Test II) groups using one-way ANOVA followed by Tukey's multiple-comparisons test. The percentage wound contraction was significantly increased in the standard, gingelly oil, and Novina Enne groups than in the control group ($p < 0.01$, $p < 0.0001$, and $p < 0.0001$, respectively). Gingelly oil and Novina Enne also showed significantly increase in wound contraction rate than Aloe vera gel ($p <0.01$ and $p < 0.0001$, respectively). However, the difference between the gingelly oil and Novina Enne groups was not statistically significant ($p = 0.2741$).

On day 16, Tukey's multiple comparisons test revealed statistically significant differences in wound contraction rate among all groups. The wound contraction rate was significantly higher in the Standard group than in the Control group ($p < 0.05$). gingelly oil and Novina Enne showed significantly higher wound contraction rates than the control group ($p < 0.0001$ for both comparisons). Furthermore, gingelly oil (Test I) and Novina Enne (Test II) demonstrated significantly higher wound contraction rates than the Standard group ($p < 0.001$ and $p < 0.0001$, respectively). Novina Enne (Test II) also showed a significantly higher wound contraction rate than gingelly oil(Test I) ($p <0.05$). Overall, the findings indicate that Novina Enne exhibited the greatest wound contraction, followed by gingelly oil group, Standard, and Control [Figure 2, Figure 3].

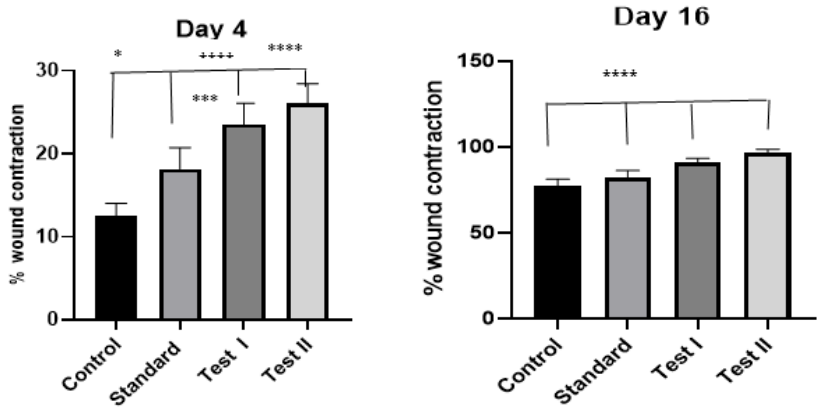


Figure 2. Effect of Novina Enne (Test II) on the wound contraction rate in the excision wound model in Wistar rats. Data were analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test. *$p < 0.05$ statistically significant; On day 4 -control vs standard **$p < 0.01$, control vs gingelly oil (Test I) and control vs test II ***$p < 0.0001$; standard vs Test I **$p < 0.01$, standard vs Test II ****$p < 0.0001$. No significance between Test I and Test II groups ($p = 0.2741$). On Day 16, Control vs Standard, $p < 0.05$); Control group vs Test I and Control group vs Test II $p < 0.0001$, standard vs Test I, $p < 0.001$; Standard vs Test II $p < 0.0001$. Test II vs Test I $p < 0.05$.



Figure 3: Image showing wound contraction rate in excision wound model treated with Novina Enne [test II]; observed in different days

Period of epithelization

The period of epithelisation was significantly shorter in the Standard, gingelly oil (Test I), and Novina Enne(Test II)groups compared with the Control group ($p < 0.0001$ for all comparisons). However, no statistically significant differences were observed between the Standard and Test I groups ($p = 0.9595$), Standard and Test II groups ($p = 0.7570$), or Test I and Test II groups ($p = 0.9595$). These findings suggest that all three treatment groups exhibited a significant reduction in the period of epithelisation compared with the c ontrol group, with no significant differences among the treatment groups [Figure 3].

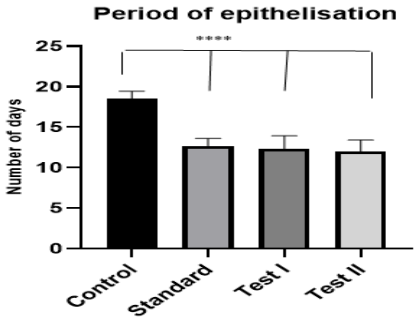


Figure 4. Effect of Novina Enne application on the period of epithelisation. Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Control vs Standard, Control vs gingelly oil (Test I), and Control vs NovinaaEnne (Test II) groups (**** $p < 0.0001$). Differences among the Standard, Test I, and Test II groups were not statistically significant ($p > 0.05$).

DISCUSSION AND CONCLUSION:

Wound healing is a complex biological process involving the restoration of damaged tissue, whereas wound contraction refers to the reduction in wound size through the inward movement of wound edges, facilitating wound closure (Gurtner et al., 2008). In the present study, topical application of Aloe vera gel (standard), gingelly oil (Test I), and Novina Enne (Test II) resulted in increased wound-breaking strength compared with the control group. Among the treatment groups, Novina Enne (Test II) demonstrated significantly greater wound-breaking strength than both the standard treatment and gingelly oil group. Similarly, in the excision wound model, Novina Enne exhibited a higher wound contraction rate than gingelly oil and the Aloe vera gel (standard) treatment group. Although all treatment groups showed a significantly shorter

period of epithelisation compared with the control group, no statistically significant differences were observed among the treatment groups.

Aloe vera gel has been investigated for its wound-healing properties (Takzare et al., 2009), with clinical studies reporting improved healing outcomes in certain wound types (Burusapat et al., 2018). Similarly, experimental studies have suggested that gingelly oil may promote wound contraction and tissue regeneration. The antioxidant activity of gingelly oil may contribute to its potential effects on wound repair by helping counter oxidative stress (Kiran and Asad, 2008). However, these findings should be interpreted in the context of the specific formulations and experimental models investigated. Traditionally prepared oil extracts have principally three components namely, a liquid which may be aqueous decoction of one or more herbs, a fine paste of the herbs and a vegetable oil. Therapeutically, Gingelly oil has traditionally been used for massage and serves as a base oil in the preparation of various therapeutic oils.

Possible mechanism of wound healing property of Novina Enne, the polyherbal oil seen in the present study, as it contains *Syzygium aromaticum* (clove flower buds), *Nigella sativa* seeds, *Rubia cordifolia* roots, *Zingiber officinale* rhizomes, *Aloe barbadensis*, raktabola resin, *Acacia catechu* bark, *Ferula asafoetida*, camphor derived from *Cinnamomum camphora*, and gingelly (sesame) oil. Several of these ingredients have been investigated individually for their potential antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties.

Syzygium aromaticum has demonstrated antimicrobial activity against organisms including Escherichia coli and *Pseudomonas* species. Its reported anti-inflammatory properties may also contribute to the regulation of inflammatory responses during wound repair (Plefh et al., 2021, Mohd Kamal, 2025, Banerjee et al., 2020)

Similarly, *Nigella sativa* seeds contain thymoquinone, a bioactive constituent associated with anti-inflammatory and wound-healing effects. Experimental studies have reported enhanced fibroblast activity, granulation tissue formation, wound contraction, and re-epithelialisation following thymoquinone treatment (Sallehuddin et al., 2020, Soltanfar et al., 2023)

Zingiber officinale rhizomes have also been investigated for wound-healing, anti-ulcerogenic, and antimicrobial activities (Cheshfar et al., 2023, Sulieman et al., 2024).

In addition, *Acacia catechu* bark extracts have demonstrated antimicrobial activity against several bacterial and fungal pathogens relevant to wound infections, including *Staphylococcus aureus, Pseudomonas aeruginosa*, E. coli, and *Candida albicans* (Negi and Dave, 2010). Extracts of *Ferula asafoetida* have been reported to enhance fibroblast viability and promote wound repair in experimental models [(Rakhymbayev et al., 2026)].

Evidence also suggests that extracts of *Cinnamomum camphora* may influence wound healing. In an experimental incision-wound model, treatment with an ethyl acetate-soluble fraction of the plant extract was associated with increased wound tensile strength. Improvements in granulation tissue weight and biochemical indicators associated with collagen and extracellular matrix formation have also been reported (Khalil et al., 2025, Sen and Garg, 2020, Sen and Garg, 2019). Camphor, containing preparations have additionally been investigated for antimicrobial activity, although the effects depend on the preparation and concentration used (Thanushiyan, 2025).

Gingelly oil, which serves as the base oil in Novina Enne, has traditionally been used for massage and as a vehicle for preparing therapeutic oils. Its lipid constituents and reported antioxidant properties may contribute to skin conditioning and protection against oxidative damage. However, the specific contribution of gingelly oil to wound healing in the present formulation requires further investigation.

Taken together, the reported biological activities of these ingredients provide a plausible rationale for investigating Novina Enne as a topical wound-healing preparation. In the present study, the observed improvements in wound contraction and wound-breaking strength suggest potential benefits of the formulation in experimental wound healing. Nevertheless, because Novina Enne contains multiple ingredients, the individual contribution of each component and the possibility of synergistic effects cannot be established from the present findings alone.

Moreover, properly prepared oils have long expiry (about one year) without any separate preservatives which helps in the easy storage of the medicinal extract. Home based oil preparation is economical and convenient. In this study, Aloe vera gel is used as a standard drug for wound healing as it is known to have wound healing and anti- inflammatory activity (Yadav et al., 2012).

The present study demonstrates the potential wound-healing activity of Novina Enne, a polyherbal oil, following topical application in experimental incision and excision wound models. The findings support its traditional use in Ayurveda for wound management and suggest that traditionally prepared polyherbal oils may have potential as wound-healing agents. However, further studies are required to elucidate the underlying mechanisms of action and evaluate the safety and efficacy of the formulation. Additional phytochemical investigations are warranted to identify and characterize the bioactive constituents that may contribute to its wound-healing effects.

LIMITATIONS OF THE STUDY

The present study has certain limitations. First, the evaluation was conducted using experimental animal wound models, and the findings may not be directly extrapolated to wound healing in humans. Second, as Novina Enne is a polyherbal formulation containing multiple ingredients, the individual contributions of its constituents and the possibility of synergistic effects could not be established. Third, the study did not investigate the specific molecular and cellular mechanisms underlying the observed wound-healing effects. Furthermore, detailed phytochemical characterization of the formulation and identification of the bioactive constituents responsible for its activity were not undertaken. Further studies involving mechanistic investigations, phytochemical analysis, and systematic safety evaluation are warranted to establish the therapeutic potential of Novina Enne.

Future Research
Further studies are needed to identify the bioactive constituents of Novina Enne and elucidate its wound-healing mechanisms. Dose-response studies, histopathological evaluation, and comprehensive safety assessments are warranted, followed by clinical studies to establish its therapeutic efficacy in humans.

CONCLUSION
The present study demonstrated the potential wound-healing activity of Novina Enne, a polyherbal oil, in experimental incision and excision wound models. Topical application of Novina Enne improved wound-breaking strength and wound contraction compared with the control group. Although all treatment groups showed a significantly shorter period of epithelisation than the control group, no significant differences were observed among the treatment groups for this parameter. These findings provide experimental support for the traditional use of Novina Enne in ayurvedic wound management. Further studies are warranted to elucidate its mechanisms of action, identify the bioactive constituents responsible for its effects, and establish its safety and efficacy.

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Ai Use Statement
We acknowledge the use of the OpenAI-ChatGPT-free version, solely for grammar correction and language refinement. The data and original ideas remain unaltered. The authors reviewed and edited the output and takes full responsibility for the final content.

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Declaration of conflict of interest
The authors declare that there is no conflict of interest.

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