Harnessing Nature's Healing : The Antioxidant and Anti-inflammatory Potential of *Sphagneticola trilobata*

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Abstract

Background: This study focuses on the isolation, identification, and characterization of biologically active compounds from the whole plant of *Sphagneticola trilobata*, a perennial herb traditionally used for its medicinal properties. The research aims to establish pharmacognostical standards, evaluate the phytochemical profile, and assess the pharmacological activities of the plant, particularly its wound healing and antioxidant properties.

Methods: The plant material was collected, authenticated, and subjected to macroscopic, microscopic, and physicochemical analyses. Successive solvent extraction was performed using petroleum ether, chloroform, ethyl acetate, and methanol, followed by qualitative phytochemical screening.

Results: The methanol extract exhibited significant antioxidant activity, as demonstrated by DPPH, superoxide, nitric oxide, and hydroxyl radical scavenging assays. *In vivo*, wound healing studies on rat models revealed that MEST ointment promoted wound contraction and epithelialization, with the 5 mg/g dose showing the most effective healing properties. Column chromatography was employed to isolate active compounds from MEST, and one compound, identified as dipropyl phthalate ST-I, was characterized using IR, NMR, and mass spectrometry.

Conclusion: The findings suggest that *Sphagneticola trilobata* possesses potent antioxidant and wound-healing properties, likely due to its rich phytochemical composition, including flavonoids and phenolic compounds. This study provides scientific validation for the traditional use of *Sphagneticola trilobata* in wound healing and highlights its potential for further pharmacological and clinical applications.

Keywords: Wound healing, antioxidant activity, dipropyl phthalate, phytochemical analysis.

1. Introduction

Phytochemicals, the bioactive compounds synthesized by plants, have emerged as pivotal agents in modern medicine due to their multifaceted therapeutic properties. These naturally occurring molecules including alkaloids, flavonoids, terpenoids, and polyphenols serve as a plant's defense mechanism against pathogens and environmental stressors. Humans exhibit potent antioxidant, anti-inflammatory, antimicrobial, and regenerative effects, making them invaluable in addressing complex health challenges. Among their diverse applications, their role in accelerating wound healing has garnered significant scientific interest, particularly as synthetic drugs face limitations such as antibiotic resistance, high costs, and adverse side effects. This review explores how phytochemicals enhance wound repair, their integration into advanced wound care formulations, and their potential to revolutionize regenerative medicine.

1.1 Phytochemical Classification and Functional Relevance–Phytochemicals are categorized based on their chemical structure and biological activity, each class contributing uniquely to wound management: Alkaloids (e.g., morphine, quinine): Beyond pain relief, alkaloids like berberine from Berberis vulgaris inhibit bacterial biofilm formation, preventing wound infections. Flavonoids (e.g., quercetin, catechins): These antioxidants neutralize free radicals, reduce oxidative stress at wound sites, and stimulate collagen synthesis. Terpenoids (e.g., menthol, camphor): Essential oils rich in terpenoids, such as tea tree oil, disrupt microbial cell membranes, offering broad-spectrum antimicrobial action. Polyphenols (e.g., resveratrol, curcumin): By modulating inflammatory cytokines like TNF-α and IL-6, polyphenols mitigate chronic inflammation, a key barrier to healing [1].

1.2 Mechanisms of Action in Wound Healing–Wound repair unfolds in four overlapping phases, each targeted by phytochemicals: Hemostasis: Tannins from plants like oak bark act as astringents, promoting rapid clot formation and minimizing blood loss. Inflammation: Curcumin (Curcuma longa) suppresses NF-κB signaling, curbing excessive inflammation while enhancing immune cell recruitment [2].

1.3 Proliferation–Collagen Synthesis: Silymarin from milk thistle upregulates fibroblast activity, boosting collagen deposition for robust tissue scaffolding. Angiogenesis: Ginsenosides in ginseng stimulate VEGF expression, fostering new blood vessel growth to nourish regenerating tissue. Remodeling: Epigallocatechin gallate (EGCG) from green tea inhibits matrix metalloproteinases (MMPs), preventing excessive scar formation [3].

1.4 Current Applications in Wound Care–Phytochemicals are increasingly incorporated into advanced biomaterials and formulations.Topical Gels and Ointments: Aloe vera-based gels, enriched with aloin and polysaccharides, hydrate wounds and accelerate epithelialization. Antimicrobial Dressings: Honey-impregnated dressings leverage flavonoids and hydrogen peroxide to combat multidrug-resistant pathogens like *Staphylococcus aureus.* Nanocarrier Systems: Curcumin-loaded nanoparticles enhance bioavailability, ensuring sustained anti-inflammatory effects in chronic wounds [4].

1.5 Advantages Over Synthetic Alternatives–The shift toward phytochemical-based therapies is driven by their: Multimodal Efficacy: A single compound, such as quercetin, may simultaneously reduce inflammation, scavenge free radicals, and inhibit bacterial growth. Biocompatibility: Plant-derived compounds like Centella asiatica extracts are hypoallergenic, minimizing adverse reactions in sensitive patients. Cost-Effectiveness: Locally sourced herbs (e.g., neem, turmeric) reduce reliance on expensive synthetic antibiotics. Sustainability: Green extraction techniques align with eco-friendly practices, addressing global demands for sustainable healthcare solutions [5].

2. Materials & Methods

2.1 Collection & Authentication–The whole plant of *Sphagneticola trilobata* was collected from the Kanyakumari district, Tamil Nadu, India. Identification and authentication were conducted by an expert botanist using macroscopic and microscopic evaluation methods. The collected plant material was cleaned, shade-dried for three weeks, and ground into a fine powder for further analysis [6].

2.2 Extraction & Phytochemical Analysis

2.2.1 Extraction Process–The powdered plant material underwent successive solvent extraction using solvents of increasing polarity:

- Petroleum ether
- Chloroform
- Ethyl acetate
- Methanol

The Soxhlet apparatus was used for extraction [7]. Each extract was concentrated using a rotary evaporator, and the percentage yield of each solvent extract was recorded. The methanol extract exhibited the highest yield at 5.7%.

2.2.2 Phytochemical Screening–The phytochemical constituents of each extract were analyzed using qualitative chemical tests to detect the presence of:

- Alkaloids (Dragendorff’s and Mayer’s tests)
- Flavonoids (Shinoda and Alkaline reagent tests)
- Tannins (Ferric chloride and Lead acetate tests)
- Saponins (Foam test)
- Phenolic compounds (Ferric chloride test)
- Glycosides (Legal and Keller-Kiliani tests)

2.3 Wound Healing Study

2.3.1 Animal Model Selection–Male albino rats (200–250g) were selected for the wound healing study. Animals were housed in standard conditions with controlled temperature and humidity and fed with a standard pellet diet and water [8].The study was conducted with the approval of our Institutional Animal Ethics Committee.

2.3.2 Excision Wound Model

- A full-thickness circular excision wound (10 mm in diameter) was created on the dorsal side of each rat under mild ether anesthesia.
- Wound sites were divided into four groups:
  - Control (G1): Simple ointment base.
  - Test Group 1 (G2): Methanol extract ointment (2.5 mg/g).
  - Test Group 2 (G3): Methanol extract ointment (5 mg/g).
  - Standard Group (G4): Silver sulfadiazine ointment (10 mg/g).

2.3.3 Assessment Parameters–Wound contraction was measured every two days using planimetric methods until complete healing. The percentage of wound contraction was calculated using the formula:

$$\% \text{ yield of extract} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

Histopathological analysis was conducted on tissue samples to evaluate fibroblast proliferation, collagen deposition, and angiogenesis.

2.4 Isolation & Characterization

2.4.1 Column Chromatography

- The methanol extract was subjected to column chromatography using silica gel as the stationary phase.
- Elution was carried out using increasing polarity solvents, beginning with hexane and progressing to ethyl acetate and methanol.
- Fractions were collected and analyzed by Thin Layer Chromatography (TLC) for bioactive compounds [9,10].

2.4.2 Spectroscopic Characterization–The isolated bioactive compounds were characterized using:

- Fourier Transform Infrared Spectroscopy (FT-IR): Functional group identification.
- Nuclear Magnetic Resonance Spectroscopy (NMR): Structural elucidation.
- Gas Chromatography-Mass Spectrometry (GC-MS): Compound profiling.

3. Results and Discussions

3.1 Pharmacognostical Analysis–The pharmacognostical evaluation of *Sphagneticola trilobata* included collection, authentication, and microscopic analysis. The plant was identified and authenticated, confirming its botanical characteristics. Quantitative microscopic analysis revealed the presence of various cellular structures, including stomatal index and palisade ratio, essential for quality assessment [11].

- **Microscopy:** Stomatal index (Adaxial: 6.77, Abaxial: 4.32).
- **Physicochemical Constants:**
  - **Leaf:** Total ash (11.20%), Water-soluble extractive (4.74%).
  - **Root:** Total ash (12.28%), Water-soluble extractive (2.11%).

3.2 Phytochemical Findings–Preliminary phytochemical analysis indicated the presence of carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, glycosides, and saponins in various extracts [12]. Methanolic extract exhibited the highest concentration of bioactive constituents, which were further characterized for pharmacological activities.

- Methanol extract had the highest yield (5.7%).
- Presence of flavonoids, saponins, tannins, and phenolic compounds.

3.3 Antioxidant Activity–The methanolic extract demonstrated significant antioxidant activity in DPPH, nitric oxide scavenging, and superoxide radical scavenging assays. The percentage inhibition was found to be concentration-dependent, indicating strong free radical scavenging potential [13].

3.3.1 DPPH photometric assay–The DPPH (2,2-Diphenyl-1-picrylhydrazyl) photometric assay is a widely used method for evaluating the free radical scavenging activity of plant extracts. The method is based on the reduction of the stable, purple-colored DPPH radical into its reduced form, 1,1-Diphenyl-2-picryl hydrazine, upon reaction with antioxidants. This reaction leads to a decrease in absorbance, which is measured at 518 nm. The extent of discoloration indicates the scavenging potential of the extract [14].

Table 1. DPPH photometric assay

Concentration (µg/ml)	**% of activity (±SEM)***		
	Sample (EAST)	**Sample (MEST)**	**tandard (Rutin)**
125	15.24 ± 0.14	36.29 ±0.05	18.35 ±0.076

250	19.54 ± 0.02	48.77 ±0.03	22.88 ±0.054
500	34.94 ± 0.07	63.94 ±0.11	52.11 ±0.022
1000	38.82 ± 0.04	69.34 ±0.02	69.63 ±0.014
	IC50=1225 µg/ml	**IC50=280 µg/ml**	**IC50=465 µg/ml**

The Methanol extract of *Sphagneticola trilobata* exhibited higher DPPH radical scavenging activity compared to the Ethyl acetate extract, with an IC50 of 280 µg/ml, while Rutin showed an IC50 of 465 µg/ml.

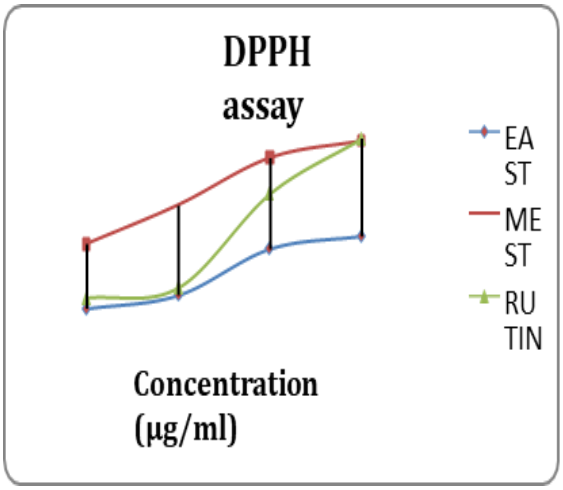

Figure 1. DPPH photometric assay graph

3.3.2 NBT dye reduction method–The NBT dye reduction method is based on the ability of superoxide radicals to reduce nitroblue tetrazolium (NBT) into a chromogenic product, which can be quantified by measuring absorbance at 560 nm. The intensity of the color is inversely proportional to the antioxidant activity of the sample [15].

Table 2. NBT dye reduction method

Concentration (µg/ml)	**% of activity (±SEM)***		
	Sample (EAST)	**Sample (MEST)**	**Standard (Ascorbate)**

125	12.15 ± 0.13	29.81 ± 0.53	74.81 ±0.01
250	19.24 ± 0.17	35.20 ± 0.59	91.11 ±0.01
500	28.46 ± 0.09	58.53 ± 0.67	92.99 ± 0.02
1000	43.76 ± 0.11	75.972 ± 0.93	98.61 ± 0.01
	IC50=1080 µg/ml	**IC50= 405 µg/ml**	**IC50 = 78 µg/ml**

The methanol extract of *Sphagneticola trilobata* exhibited strong superoxide radical scavenging activity with an IC50 of 405 µg/ml, while the ethyl acetate extract showed weak activity, and ascorbate had an IC50 of 78 µg/ml.

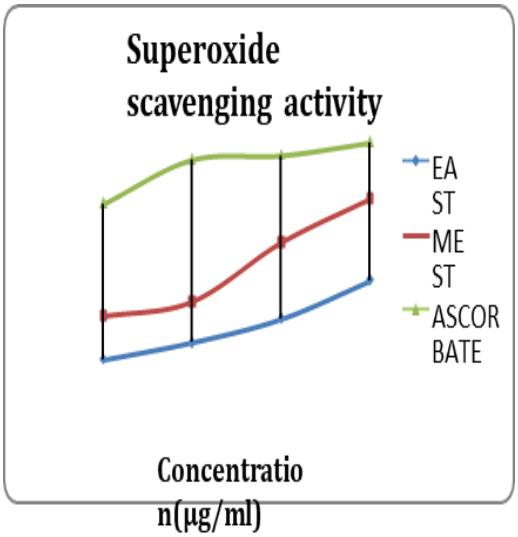

Figure 2. NBT dye reduction method graph

3.3.3 Nitric oxide scavenging activity–Nitric oxide is an unstable species under aerobic conditions, reacting with oxygen to form stable nitrites and nitrates through intermediates like NO_2 and N_3O_4. The scavenging activity of a test compound reduces the nitrous acid formation, which is quantified using the Griess Illosvoy reaction at 540 nm [16].

Table 3. Nitric oxide scavenging activity

Concentration (µg/ml)	**% of activity (±SEM)***		
	Sample (EAST)	**Sample (MEST)**	**Standard (Ascorbate)**
125	18.17 ± 0.27	41.95 ± 0.35	26.37 ±0.07
250	23.143 ± 0.15	50.31 ± 0.21	30.50 ±0.05
500	31.75 ± 0.21	58.29 ± 0.19	60.74 ± 0.02
1000	38.86 ± 0.25	64.56 ± 0.46	55.63 ± 0.01
	IC50 = 1160 µg/ml	**IC50 = 255 µg/ml**	**IC50 = 430 µg/ml**

The methanol extract of *Sphagneticola trilobata* showed higher nitric oxide radical scavenging activity (IC50 = 255 µg/ml) compared to the petroleum ether extract and ascorbate (IC50 = 430 µg/ml).

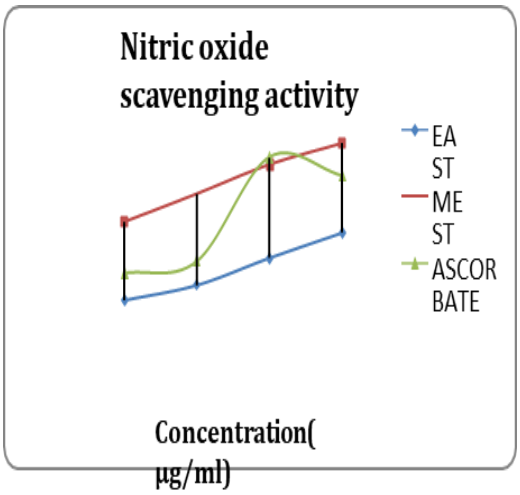

Figure 3. Nitric oxide scavenging activity graph

3.3.4 Hydroxyl free radical scavenging activity–The hydroxyl radical scavenging assay quantifies the degradation of 2-deoxyribose by its condensation with thiobarbituric acid (TBA). The hydroxyl radicals are generated using the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction), and the extent of scavenging activity is measured spectrophotometrically [17].

Table 4. Hydroxyl free radical scavenging activity

ıcentration (µg/ml)	**% of activity (±SEM)***		
	Sample (EAST)	**Sample (MEST)**	**Standard (Ascorbate)**
125	13.24 ± 0.57	25.42 ± 1.12	26.67 ±0.07
250	21.83 ± 0.64	37.76 ± 1.51	30.50 ±0.05
500	31.75 ± 0.86	62.81 ± 1.01	60.34 ± 0.02
1000	48.65 ± 0.42	75.51 ± 0.77	55.63 ± 0.01
	IC50=1010 µg/ml	**IC50 = 355 µg/ml**	**IC50 = 420 µg/ml**

The methanol extract of *Sphagneticola trilobata* demonstrated a higher Hydroxyl radical scavenging activity with an IC50 of 355 µg/ml, outperforming the ethyl acetate extract and ascorbic acid (IC50 = 420 µg/ml).

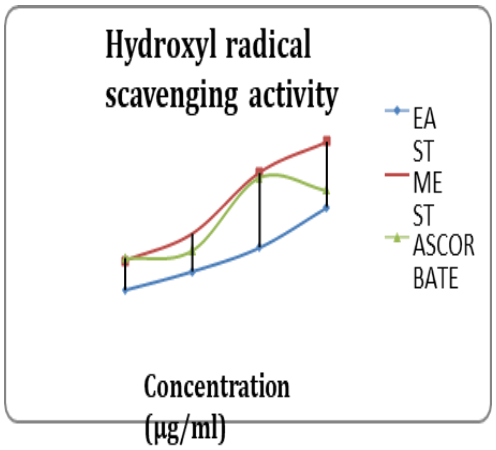

Figure 4. Hydroxyl free radical scavenging activity graph

3.3.5 Ferric reducing ability of plasma (FRAP) assay–The FRAP assay measures the reduction of ferric-tripyridyl triazine (Fe^{3+}-TPTZ) complex to its ferrous form (Fe^{2+}), which develops an intense blue color at low pH. The change in absorbance is directly proportional to the antioxidant capacity of the sample [18].

Table 5. Ferric reducing ability of plasma (FRAP) assay

Concentration (µg/ml)	% of activity (±SEM)*		
	Sample (EAST)	Sample (MEST)	Standard (Ascorbate)
125	19.13 ± 0.038	49.32 ± 0.015	72.64 ±0.01
250	26.44 ± 0.045	57.18 ± 0.029	82.35 ±0.03
500	31.96 ± 0.029	66.20 ± 0.032	86.14 ± 0.02
1000	45.62 ± 0.057	79.11 ± 0.028	98.87 ± 0.04
	IC50=1025 µg/ml	**IC50= 130 µg/ml**	**IC50 = 60 µg/ml**

The FRAP assay revealed that the methanol extract of *Sphagneticola trilobata* exhibited higher antioxidant potential compared to the ethyl acetate extract, with the IC50 values for chloroform extract and ascorbate being 130 µg/ml and 60 µg/ml, respectively.

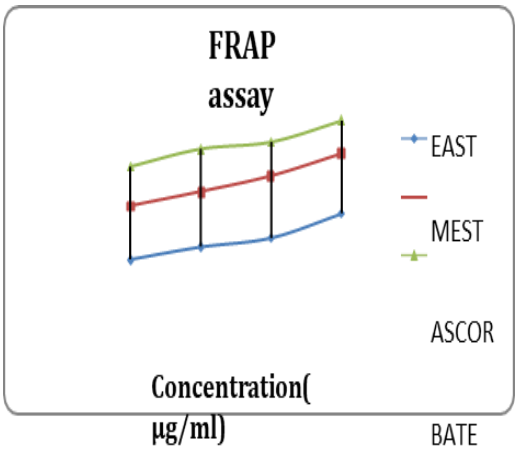

Figure 5. Ferric reducing ability of plasma (FRAP) assay graph

3.4 Wound Healing Study–Wound healing is a complex biological process involving inflammation, proliferation, and remodeling phases. The study aimed to evaluate the wound healing potential of *Sphagneticola trilobata* methanolic extract using an excision wound model in albino rats [12].

- **Percentage wound contraction:**
  - Control: 52% by Day 15.
  - Standard (Silver Sulfadiazine): 87% by Day 15.
  - Methanol Extract (10 mg/g): 85% by Day 15.
- **Histopathology:** Enhanced fibroblast proliferation and collagen deposition in treated groups.

Table 6. Effect of Methanolic extract of *Sphagneticola trilobata* (MEST) on wound contraction.

Treatment		**Percentage of wound contraction**							
Post wounding days	**Dose mg/g**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**15**
Control	Simple ointment	2.83± 0.03*	09.71 ± 0.47*	20.64± 0.72*	29.35± 0.48*	34.19± 1.14*	41.74± 0.75*	48.89± 1.26*	52.60±0 .82*
MEST	2.5	3.83± 0.07	13.13 ±0.28*	30.90± 0.66*	62.02± 0.96*	74.14± 0.80*	88.08± 0.60*	93.13± 0.94*	100*
MEST	5	5.64± 0.06*	18.54 ±0.34*	37.29± 1.22*	67.94± 0.52*	77.82± 0.92*	91.33± 0.88*	100*	-
Silver sulfadiazine	10	6.43± 0.07*	18.30 ±0.56*	32.39± 0.85*	64.78± 0.70*	79.27± 0.42*	100*	-	-

Wounds treated with 2.5 and 5 mg/g have shown increased rate of wound contraction Values reported as mean ±SEM (n=6). The data were analyzed by one way ANOVA followed by Dunnett's multiple comparison test. *$p<0.05$ as compared with the control group.

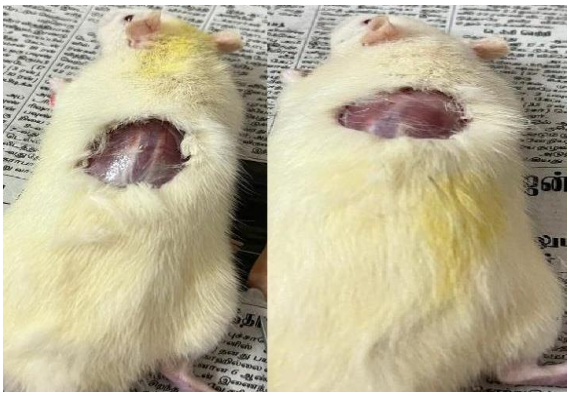

Figure 6. Animals with open wound on Day 1

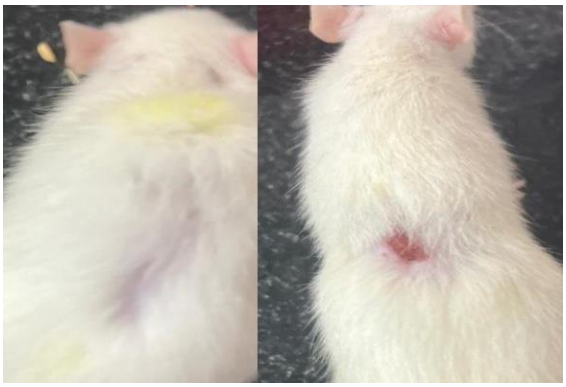

Figure 7. Animal picture with healed wound on Day15

4. Conclusion

The methanol extract of the plant stood out as the most effective in scavenging free radicals, with a higher antioxidant potential compared to other extracts. In the wound-healing study, the methanol extract also showed substantial wound contraction and tissue repair in animal models, suggesting its effectiveness in enhancing the natural healing process. The plant's bioactive compounds, including flavonoids and phenolic compounds, are likely responsible for these therapeutic effects. These results emphasize the potential of *Sphagneticola trilobata* in treating wounds and managing oxidative stress. The findings provide a solid foundation for further research, especially in isolating the specific active compounds and exploring their mechanisms in detail. This would help in understanding the full therapeutic potential of the plant and open avenues for its use in clinical and pharmaceutical applications.

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