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Formulation and Evaluation of Herbal Gel for Wound healing Application

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Abstract

Daily existence involves Daily existence involves wounds, which are frequent. Wounded tissue results from physical, chemical, mechanical, microbiological, and immunological injuries to the tissue. These injuries cause physical impairments and productivity losses. Humans naturally heal wounds by rebuilding and regenerating tissue. The highest yield was from Cissus quadrangularis and Plumeria acuminata methanol extract. Ethyl acetate extract of Cissus quadrangularis and methanol extract of Plumeria acuminata had the greatest total phenolic, flavonoid, and DPPH-estimated free radical scavenging activities. Based on these activities, extracts were chosen for HPTLC and marker compound separation. Characterization of isolated chemicals by IR, NMR, and MS. Gallic acid from ethyl acetate extract and quercetin from methanol extract of Plumeria acuminata were predicted compounds.

Keywords: Wound, Gel, Herbal

Introduction

Daily existence involves wounds, which are frequent. Wounded tissue results from physical, chemical, mechanical, microbiological, and immunological injuries to the tissue. These injuries cause physical impairments and productivity losses. Humans naturally heal wounds by rebuilding and regenerating tissue. Since wounds are distinctive, they need unique treatment. Thus, wound healing is an old medical concern. Good wound healing requires excellent scar tissue, functional and cellular repair, and wound contraction or closure. Diabetes mellitus makes wound healing difficult. Most diabetics have trouble with wound infections.

Dressing, painkillers, anti-inflammatories, corticosteroids, topical and systemic antimicrobials, and other healing agents are used to treat wounds. Advanced pharma research makes different pharmaceutical formulations for wound healing available, but high cost and adverse effects make their usage difficult.

Silver sulfadiazine, Silver nitrate, Neosporin, Mafenide, Povodine-iodine, Nitrofurazone, Oxacillin, Mezlocillin, and Gentamicin are widely used to treat severe and chronic wounds. Overuse, misuse, or widespread prophylactic use of these antibiotics can lead to drug resistance in bacteria like Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, and Klebsiella sp., which can cause wound infections. This hinders chronic wound antibiotic therapy. However, synthesized antimicrobials are poisonous to fibroblast cells, induce skin rashes, and have one pharmacological function. Plants are natural, renewable, inexpensive, readily accessible, low-impact, and multifunctional, thus they should be evaluated to eliminate these limits.

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Natural products have long been used for skin wound treatment owing to their anti-inflammatory, antibacterial, antioxidant, and cell-stimulating characteristics. Animal models and people have been used in experiments to assess these drugs' therapeutic effectiveness. These natural compounds need unique extraction, purification, quality control, and treatment processes for scientific approval. A good wound healing agent should heal wounds quickly, minimize infection, have good antimicrobial action, be cost-effective, low-toxic, non-allergenic, stable, long-lasting, effective in all wound types, reduce pain and discomfort, and restore damaged tissue's cellular structure. Wound infection is common in underdeveloped and wealthy nations owing to inadequate hygiene. Chronic wounds are a major concern for both patients and healthcare providers because they shorten life expectancy and increase the likelihood of hospitalization. According to the World Health Organization (WHO), traditional medicines are used to treat illnesses by about 80% of people worldwide. Traditional medicine hazards and benefits are supported by WHO. About 6 million people worldwide have chronic wounds.

Humans have historically used plants and their components to cure and prevent diseases. Most medicines are plant-based and made from crude extracts and combinations. Herbs are found across India. They are widely accepted as anti-diabetic, anti-arthritic, liver-protective, cough suppressant, memory enhancer, and other natural and safe medications. Today, scientists are isolating active chemical compounds and modifying them to create plant-based medications that may treat numerous disorders. Several plants have been tested to treat wounds with little side effects and maximal efficacy and cost-effectiveness. Several plants have succeeded in this endeavor. These plant-based compounds could be used by pharmaceutical companies to make new drugs for wound care. These plant-derived compounds—alkaloids, essential oils, flavanoids, tannins, terpenoids, saponins, and phenolics—have therapeutic effects on the body.

Current biomedical sciences and traditional herbal therapy worldwide are focusing on developing better and more effective wound healing agents. India has valuable knowledge about lesser-known and unknown wild plants with wound-healing properties. Ancient people recommended many herbs for wound treatment.

The aim of the present study is to develop and evaluate a herbal gel formulation using natural ingredients for effective wound healing, emphasizing safety, efficacy, and ease of application.

Objectives

- Develop a stable and biocompatible herbal gel formulation using natural plant extracts with proven wound-healing properties.
- Evaluate the physical characteristics of the gel, such as texture, viscosity, pH, and spreadability, to ensure usability and consistency.
- Conduct in vitro studies to assess the antimicrobial, anti-inflammatory, and antioxidant activities of the herbal gel.
- Perform in vivo studies to evaluate the wound-healing efficacy and rate of tissue regeneration in suitable animal models.
- Assess the safety of the gel through skin irritation and cytotoxicity studies to ensure its suitability for topical application.

Justification of The Study

Although healing is a component of the human defense system that restores the structure and function of the skin without external support, improper, poor, or delayed healing encourages microbial infection, which can aggravate the wound, result in complications, and cost the economy and health of both developed and developing nations. Ideal healing involves heamostasis, inflammation, proliferation, collagenation, granulation, cell migration, re-epithelization, and remodeling. Any disruption in this cascade may slow skin healing. Diabetes still makes it hard to heal wounds, which can lead to gangrene or death. The pharma industry offers topical and systemic antibacterial, pain-relieving, anti-inflammatory, and antiseptic wound treatments. Gentamicin, neosporin, bacitracin, nitrofurazone, silver sulphadiazine, chlorhexidine, etc. are used topically. They cure wounds well, but they can cause microbial resistance, allergic reactions, rashes, and expensive production costs. Overuse, abuse, and prophylactical use of

microbiological agents cause medication resistance. Antiseptics injure microbial and host cells, thus they should be used sparingly. To address these constraints, researchers worldwide are exploring safe, clinically effective, low-cost, and tolerable alternatives to existing medications.

The World Health Organization (WHO) continually encourages research into the medicinal properties of plants. Plants include bioactive phytoconstituents that may treat and start manufactured medications. Tribes and people have used plant paste, juice, extract, etc. to cure wounds for a long time. Plant secondary metabolites including tannins, triterpenoid, and alkaloids may impact healing cascade stages. Due to a lack of evidence regarding their efficacy and safety, these plant extracts and metabolites cannot be purchased in stores. Standardizing and validating them scientifically using advanced methods is necessary to identify and establish them as therapeutic agents worldwide.

Material and Methods

Table 3.1: List of material

No	Chemicals/Products	Company Name	Location
1	Rutin-Ciocalteu reagent, Folin-Ciocalcetide, Potassium chloride, Rutin-Merck	S.D. Fine Chemicals	Mumbai, India
2	Medicare and Betadine	Not mentioned	Not mentioned
3	Ketamine	Troikka Ltd.	Not mentioned
4	Aluminum chloride, water, sodium bicarbonate, sodium hydroxide, petroleum ether, chloroform, ethyl acetate, methanol, citric acid	S.D. Fine Chemicals	Pune, India
5	Sodium carbonate, wool fat, cetosteryl alcohol, hard paraffin	S.D. Fine Chemicals	Pune, India
6	Dithiobis nitro benzoic acid (DTNB), potassium acetate, carrageenan, silica gel	Media Industry	Mumbai, India
7	Thiobarbituric acid (TBA), butylated hydroxyanisole (BHA)	Media Industry, S.D. Fine Chemicals	Mumbai, India

Table 3.2 List of Equipment

S. No.	Name of Equipment	Manufacturer/Model No.
1	Electronic Weighing Machine	Wensar PGB-200-W
2	Digital pH Meter	Elico Limited, Hyderabad, LI120
3	Spinot and Electromagnetic Stirrers with Hot Plates	Tarson 6020
4	Ultraviolet Spectrometer	Shimadzu-PharmaSpec-1700
5	Rotating Vacuum Evaporator	Manufacturer not specified, Mumbai, India
6	Plethysmometer	Medicaid, Chandigarh
7	HPTLC Software	CAMAG, Linomat5, WinCats
8	Time-of-Flight Infrared Spectroscopy	Manufacturer not specified

Because the purity and quality of phytoconstituents are affected by the season, collecting plants is essential for research. The location of plant or part collection might also impact phytoconstituents. This variance is caused by soil system, climatic circumstances, ecological elements including temperature, light, rainfall, altitude, and others. Plants with a lot of active phytoconstituents can yield crude pharmaceuticals that may be affected by the weather.

Collection, Authentication, Extraction & Preliminary Phytochemical Screening

Indira Nagar, near C.S.J.M. In March, Plumeria acuminata leaves and fresh, robust stems of Cissus quadrangulais L. were produced at the University

of Kanpur. Water was used to wash plant materials after collecting to remove dirt and foreign contaminants.

Extraction, Isolation, Separation And Characterization of Phytocomponents

Extraction is necessary to remove phytoconstituents from plants or their components for further study to determine their therapeutic worth. A thorough literature review revealed that plant extracts or components may be medicinal. Soxhlet extraction, a repeated extraction, works well for sparingly soluble components and is quick and continuous. This research used soxhlet extraction since it has several benefits.

Extraction Process

Plants were dehydrated in shade for 30–50 days after collection and identification. Plumeria acuminata leaves and Cissus quadranguaris stems were dried and ground into coarse powder. These air-dried powdered drugs were soxhlet extracted with Pet ether, chloroform, ethyl acetate, and methanol in Soxhlet apparatus at 60°C for 8–10 hours or until the siphon showed colorless liquid. p A 1-liter soxhlet apparatus was filled with 100 gm of crushed medication and defatted with 250 ml Petroleum ether (40-60°C).

The extraction proceeded until the siphon solvent was clear. To test full defatting, a drop of extracted material was put on filter paper and no oilydrop appeared. After defatting, the extract was filtered and the solvent separated by rotational vacuum evaporation at 50°C. The residue was placed on a petri dish and desiccated over anhydrous sodium sulphate. % pet ether extract yield was determined. Defatted exhaust was dried naturally at room temperature and extracted with chloroform, ethyl acetate, and methanol.

The exhausted material was naturally dried before extracting with the next solvent. For preliminary phytochemical and pharmacological screening, rotary vacuum evaporator-concentrated extracts were refrigerated in airtight containers. We repeated the extraction process to get crude extracts for future research.

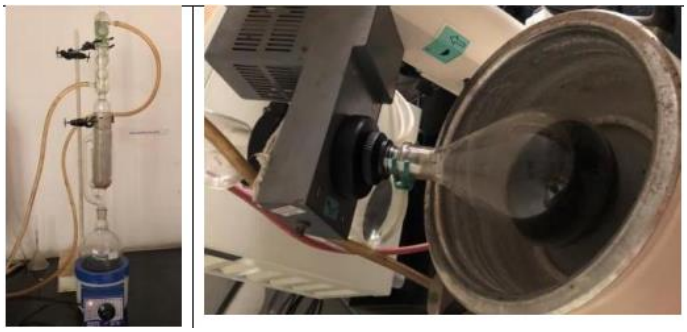

Fig 3.1 The Soxhlet Extractor and the Rotary Vacuum Evaporator are used to concentrate the extract after extraction.

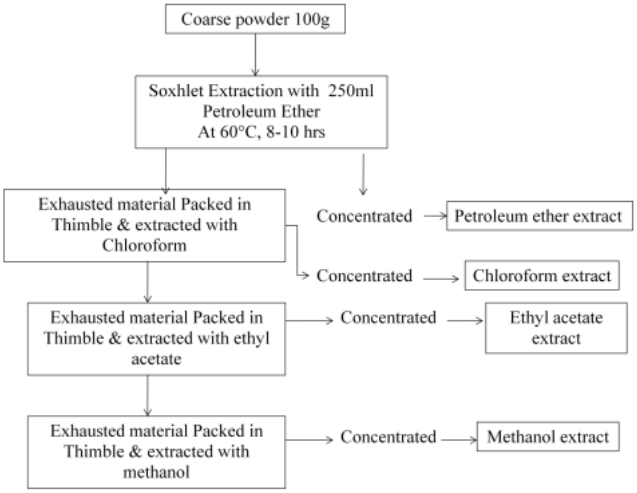

Figure 3.2 For extraction flow diagram

Preliminary Phytochemical Screening

Qualitative test Analysis

To detect phytoconstituents such flavonoids, alkaloids, tannins, phenolic compounds, sterols, terpenoids, carbohydrates, etc., all dried extracts were chemically tested.

Testforalkaloids

- **Mayer'sReagent:** Extract +0.2 mldiluteHCl+0.1 mlMayer's reagent produced white or yellow precipitates indicating alkaloid content.
- **Dragendroff'sreagent:** The orange-brown precipitate from extract+0.1mlDragendorff'sreagent+0.1ml HCl proved alkaloid presence.
- **Wagner'sreagent:** Alkaloid-containing brown precipitates were extracted using 0.1ml of HCl and Wagner's reagent.
- **Hager's reagent:** Extract + 0.2 ml dilute HCl + 0.1 ml Hager's reagent (saturated picric acid)—yellow precipitates proved alkaloid presence.

Test for glycosides

- **Killer Killani test:** 1 ml extract + 0.5 ml glacial acetic acid + 2-3 drops 1% aq. The brown ring at the interface of FeCl3solution suggests cardiac glycosides.

Brontrager's test: After extracting +3 ml benzene, filter. Filtrate +5 ml 10% ammonia solution—pink, violet, or crimson bottom phase indicates free anthraquinone.

Salkowski's test: A slow shake of the extract, 2 milliliters of chloroform, and 2 milliliters of HCl revealed a reddish-colored steroid.

Liebermann's test: Extract+ 2 ml CH3COOH + 2 ml chloroform+ concentrated HCl—green hue revealed terpenoidal glycoside aglycon unit.

Test for Tannins

- **Bromine watertest:** Decoloration of water from extract and little amount of bromine verified tannins..
- **Ferricchloride test:** 500mg dry extract + little water + boiling. Cooled filtrate + a few drops 0.1% FeCl3-- The deep blue-black hue suggested tannins.
- **Gelatin Test:** Extract + gelatin solution (1%) containing 10% NaCl produced white precipitate, indicating tannin.

TestForFlavonoids

Shinoda test: Extracts + alcohol + little amount Mg + concentrated HCl acid--magenta hue proved flavonoids.

HPTLCFingerprintingProfileforCQethylacetate fraction

To identify fraction chemicals, HPTLC fingerprinting was done. A CAMAG (Germany) HPTLC apparatus was used. Applying 5 µL of 10 mg/ml sample on precoated silica gel G F254 plates (Merck) using CAMAG Linomat 5 applicator. The 7:5:1 solvent system detected gallic acid. Toulene: ethyl acetate: formic acid The CAMAG twin trough chamber, which was saturated with mobile phase, was used for plate development. CAMAG scanner 3 and Win CATS scanned the developed plate at 254 and 366 nm after drying and visualizing it in a U.V. Cabinet.

Conditions for HPTLC Fingerprinting Profile

10 mg/ml sample preparation; Linomat 5 Applicator (Camag), inert spray gas, Sample solvent: Methanol, Dosage speed: 150nl/s, Predosage volume: 0.2µl, Application Positio: 8.0mm, Band Length: 6.0mm; Development: Presaturated Camag Twin trough chamber 10x10cm, Mobile Phase: Toulene: Ethyl acetate: Formic acid 7:5:1; Detection: Camag Scanspeed 100nm/s, Lamp D2 & W, Start wavelength 200nm, End wavelength 700nm, Software Win CATS Planar Chromatography Manager.

HPTLCFingerprintingProfileforPAmethanolextract

To identify fraction chemicals, HPTLC fingerprinting was done. A CAMAG (Germany) HPTLC apparatus was used. Applying 5 µL of 10mg/ml sample to precoated silica gel G F254 plates (Merck) using CAMAG Linomat 5 applicator. The quercetin detection solvent system was toulene:acetone:formic acid 4:5:1. Plates were developed in CAMAG twin trough chambers saturated with mobile phase. After drying and observing in a UV, the developed plate was scanned at 254 and 366 nm by the CAMAG scanner 3 and Win CATS, respectively. Cabinet. Sample preparation-10mg/ml; Linomat5 Applicator (Camag) settings, Spray inert gas, Sample solvent: Methanol, Set dosage speed to 150nl/s, predose volume to 0.2µl, application position to 8.0mm, and band length to 6.0mm. Development: Presaturated Camag Twin Trough Chamber 10x10cm, Mobile Phase - Toulene: Acetone: Formic acid 4:5:1, Camag TLC scanner, 100 nm/s fast scanning, Lamp-D2 and W Win CATS Planar Chromatography Manager, 200-700 nm wavelengths

Spreadability

Spreadability was assessed by spreading extra formulation sample between two slides and compressing it for a specified duration to achieve equal thickness. How long it took both slides to separate was deemed spreadability.

Spreadability was better between two slides that were separated for less time. Spreadability was calculated using this formula.

S =Mx L/T

S = Spreadability, M = Load on top slide, L = Glass Slide Length, T = Time (in seconds) to separate.

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Extrudability

Extrudability test estimates the strength required to eject materials from a collapsible container under weight. The amount ejected increases the formula's extrudability. Formulation was placed in a collapsible tube. When medication ejected 0.5 cm of ribbon in ten seconds, its extrudability was measured in grams. This exam was repeated three times to generate an average.

Diffusionstudy

Drug release was studied using diffusion cells. Ointment was uniformly applied on cellulose membrane's top. This cellulose membrane separated the diffusion cell's donor and receptor chambers. The receptor compartment was filled with phosphate buffer, pH 7.4, and the experimental unit was shaken magnetically to keep the temperature at 37 °C0.5. To prevent dryness, 300 mg of ointment was equally applied over the donor compartment membrane and covered with aluminum foil. Aliquots were withdrawn and drug content was measured at 275 nm by U.V.Spectrophotometer after one hour.

Loss on drying (LOD)

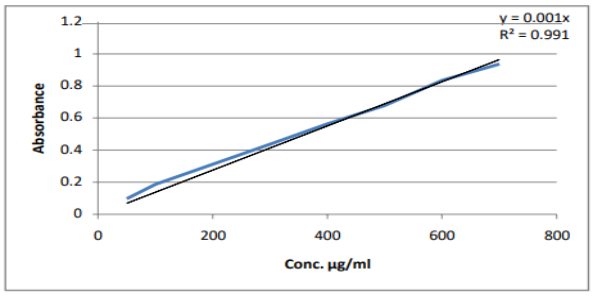
A petri-dish formulation was placed on a waterbath and dried at 105°C to measure LOD.

NonirritancyTest

This non-irritancy test used a little amount of ointment on the skin to assess its impact.

StabilityTest

Ointment physical stability was tested at 2°C, 25°C, and 37°C for one month.



EstimationofAntioxidants

To measured Superoxide dismutase (SOD),Catalase (CAT) and reduced Glutathione (GSH) level in granulated tissue, after 15 days of post wounding,wound tissue were excised and collected in phosphate buffered saline (pH7) and procedure followed according to Shuka et al, 1997, Ponrasu T et al;2013.

Histopathological study

To study histopathology, tissue was excised, preserved in 10% neutral buffered formalin, embedded in paraffin wax, cut into 5µm sections, mounted on slides, stained with hematoxylin and eosin solution, and examined under a microscope at 100 or 400 x magnification.

Statistical Analysis

Graph Pad Prism 5.00 for Windows (San Diego, CA, USA) and Excel 2007 were used for statistical analysis. Raw data from several groups were presented as mean ± SEM. Significant values were 0.05 or less. Data analysis included one-way ANOVA and Benferroni post hoc test.

Conclusion

WHO estimates that 80% of the world relies on medicinal plants for basic health care, most of which use crude plant extracts and phytoconstituents. These natural medicines may heal numerous ailments, therefore medicinal plants may be useful for developing new pharmaceuticals. Cissus quadrangularis (CQ) stems and Plumeria acuminata (PA) leaves were used to evaluate wound healing. For the sequential extraction, pet.ether, chloroform, ethy acetate, and methanol were used. Initial phytoconstituent estimation in diverse extracts. The highest yield was from Cissus quadrangularis and Plumeria acuminata methanol extract. Ethyl acetate extract of Cissus quadrangularis and methanol extract of Plumeria acuminata had the greatest total phenolic, flavonoid, and DPPH-estimated free radical scavenging activities. Based on these activities, extracts were chosen for HPTLC and marker compound separation. Characterization of isolated chemicals by IR, NMR, and MS. Gallic acid from ethyl acetate extract and quercetin from methanol extract of Plumeria acuminata were predicted compounds.Topical CQEA and MEPA ointments of 5% and 10% w/w were tested for wound healing. Both strengths of ointments showed no acute skin toxicity and steady physiochemical characteristics. Although inflammation is a defense mechanism that helps heal by removing harmful stimuli and promoting healing, excessive or uncontrolled inflammation can delay healing and exacerbate existing issues. It is vital to wound development.

In a carageenan-induced paw edema model, 250 and 500 mg/kg b.wt of ethylacetate extract of Cissus quadrangularis and methanol extract of Plumeria acuminata were tested for their anti-inflammatory properties based on acute oral toxicity. Both extracts demonstrated considerable antinflammatory effect, although the maximum dose500 mg/kg b.wt reduced paw edema volume more. The excision, incision, and burn wound models were created to evaluate the topical wound healing activities of chosen substances. Results showed that both extracts decreased wound contraction area and epithelization time dose-dependently. Inexcision wound model demonstrated 96.07 percent healing with 10% w/w ointment of CQEA, comparable to conventional treatment (98.14%). MEPA revealed 90.11% wound contraction. 5 % w/w CQEA and MEPA ointment demonstrated 87.56% and 84.95 % wound contraction at day 15 and shortened epithelization time. Stretch bearing strength of healed tissue determined from wound healing potential in incision wound model. Tensile strength was measured on the 10th day of the trial using wound rupture. Wounds treated with basic ointment base had a minimum tensile strength of 348.9 gm, whereas wounds treated with other formulations exhibited a substantial ($p<0.001$) increase in strength compared to 10% ointment of each extract increased tensile strength more than 5%. The strength of the methanolic fraction of Plumeria acuminata at 5% was the lowest of all formulation groups (483.3 gm), but it was significantly higher than the control group. Standard treated group had the maximum strength (578.8 gram). Hydroxyproline, an amino acid found in collagen, is a biomarker of wound healing because it strengthens and supports healing tissue. The tensile strength of incision wounds increases with hydroxyproline concentration. Hydroxyproline levels were significantly higher in the standard and extract-treated groups ($p<0.001$), whereas the control group had the lowest concentration (38.9 mg/g tissue). This research found that extract-treated groups' tensile strength increases dose-dependently with hydroxyproline concentration.

Burn wound model showed continuous wound contraction throughout the study, with minimum (76.31%) wound contraction in F3 (PAME5%) group, similar to control group (72.05%), and maximum (93.36%) in F2 (CQEA 10%) group, similar to standard treated group 95.8%. The epithelization period decreased according to wound contraction. Burns caused skin damage and free radical activity. GSH, SOD, and CAT are antioxidant enzymes, however burning or damage reduces their free radical neutralization.

In this investigation of homogenized granulation tissue samples from burn wound model treatment groups, 10% ointment of both plant extracts increased antioxidant status more than 5%.

Cells are damaged by free radicals, which hinder wound healing. Topical use of phytoconstituents such flavonoids and tannins promoted wound healing via early wound contraction and reduced epithelization time by inhibiting oxidative tissue damage. Flavonoids and phenolic chemicals are common phytoconstituents in plants and can cure many disorders. Due to their hydroxyl group, flavonoids are antioxidants. They are antioxidants of natural origin and treat cardiovascular, cancer, and neurological diseases. By disrupting cellular activities, reactive oxygen species from any damage may cause inflammation, myocardial infarction, and cancer. Studies show that flavonoids (quercetin) and phenolic compounds (gallic acid) have antioxidant and anti-inflammatory properties that may help wounds heal since free radicals and inflammation are key**.**

The current study found that plumeria acuminata and ethayl acetate, two methanolic extracts of plumeria, have similar wound-healing properties. Due to their high concentrations of phenolic and flavonoid components, these extracts were selected for further investigation after careful consideration. Antioxidant studies in both vitro and vivo confirmed that the plant extracts were effective. In a dose-dependent way, these extracts also showed anti-inflammatory efficacy. Extracts demonstrated impressive wound healing potential in various wound models, nearly matching that of standard drugs, according to high-performance thin-layer chromatography (HPTLC) analysis. This was due to the presence of flavonoids and phenolic compounds, which had antioxidant and anti-inflammatory properties, respectively. Plants, according to our research, have the

potential to be useful, safe wound healing agents for both humans and the pharmaceutical business.

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