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Unveiling the therapeutic potential of *Plumeria pudica*: GC-MS Analysis, phytochemical insights, and histopathological evaluation for Wound Healing and Anti-Diabetic applications

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ABSTRACT

The present study investigates the therapeutic potential of *Plumeria pudica* leaf extract, examining its efficacy as an ointment and herbal formulation for promoting wound healing in albino Wistar rats. The extract's antiseptic and anti-inflammatory properties were evaluated in its application for wound management. This study sought to evaluate the wound-healing efficacy and phytochemical profile of the ethanol extract of *Plumeria pudica*, employing preliminary screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify its bioactive compounds. The ethanol extract of *Plumeria pudica* (EEPP) ointment was formulated using a combination of wool fat, hard paraffin, white soft paraffin, polyethylene glycol, sorbitol monooleate, liquid paraffin, Span-60, Tween-60, methyl hydroxybenzoate, and propyl hydroxybenzoate to create both a herbal mixture and a topical ointment. The wound healing process was monitored over 20 days in excision wound models in rats. The phytochemical composition of the *Plumeria pudica* extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). When treated with a herbal formulation and an ointment containing 5% and 10% *Plumeria pudica* leaf extract, the excision wound closure rates reached 93.81% and 96.43% by the 14th day of evaluation. On the 20th day post-wounding, the tensile strength of the skin treated with povidone-iodine ointment was 14 ± 3.15 g, whereas rats treated with the topical herbal ointment exhibited tensile strengths of 24 ± 5.11 g and 28 ± 4.05 g. Additionally, GC-MS analysis revealed favourable skin permeability and absorption rates, indicating that the active compounds may contribute to enhanced wound healing. The topical herbal formulation and ointment containing *Plumeria pudica* leaf extract hold potential for clinical applications in wound healing and diabetes management.

Keywords – *Plumeria pudica*, Herbal Formulation, *In Vivo* Wound Healing Evaluation, GC-MS Analysis.

Introduction

Over the past decade, significant advancements have been made in understanding wound healing mechanisms.[1] The inflammatory, proliferative, and remodelling phases of wound healing are characterized by highly coordinated interactions among various cellular components, including blood vessels (platelets, macrophages, neutrophils, endothelial cells, and smooth muscle cells)[2], the epidermis (keratinocytes, melanocytes, and Langerhans cells), adnexal structures (outer root sheath cells and hair dermal papilla cells), the dermis (fibroblasts and myofibroblasts), neurons, and subcutaneous adipose tissue (adipocytes)[3]. Macrophages play a crucial role in the early, transient inflammatory phase of wound healing by executing proinflammatory functions such as phagocytosis, antigen presentation, and the production of growth factors and inflammatory cytokines that facilitate the healing process.[4] Angiogenesis is the biological process through which new blood vessels develop from pre-existing vasculature, playing a crucial role in tissue regeneration and wound healing.[5] Although angiogenesis is essential during embryonic development, it is rarely observed in healthy individuals outside of pathological conditions, inflammatory responses, the female reproductive system, and wound healing.[6] However, chronic wounds fail to undergo this process, and without identifying the underlying causes of impaired healing, even the most experienced physicians may find them challenging to treat.[7] The inflammatory mediators that impede wound healing[8] include tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), prostaglandins (PGs), reactive oxygen species (ROS), and reactive nitrogen species (RNS).[9] These mediators can interfere with wound contraction, resulting in delayed wound closure, impaired angiogenesis, and the inhibition of collagen synthesis.[10] In diabetes, elevated blood sugar levels can trigger the excessive production of proinflammatory cytokines, such as TNF-α and IL-1β. This process reduces blood flow to the wound, impairs oxygen and nutrient delivery, and inhibits collagen synthesis, ultimately delaying the healing process.[11] Antibiotic resistance is currently the most significant global threat to the effective treatment of bacterial infections. Numerous review articles have highlighted its detrimental impact on clinical and therapeutic outcomes.[13] The consequences of antibiotic resistance range from treatment failure and reliance on more expensive and potentially less effective alternative

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medications to increased morbidity and mortality rates, prolonged hospital stays, and escalating healthcare costs.[14] *Plumeria pudica* (Apocynaceae), an ornamental flowering plant closely related to the common frangipani, is commonly known as the bridal bouquet.[15] Native to Colombia, Venezuela, Panama, and India, *Plumeria pudica* is rich in phytoconstituents and exhibits various biological benefits, including anti-inflammatory and anti-diarrheal properties.[16] Several studies have reported that *Plumeria pudica* proteins exhibit anti-inflammatory effects by reducing carrageenan-induced paw oedema.[17] Therefore, this study aimed to evaluate whether incorporating *Plumeria pudica* leaf extract into a topical ointment and an oral formulation could enhance the wound healing process in an experimental animal model.

Material and Methods

Collection and Authentication

Plumeria pudica plants were collected in October 2021 from Araku Valley, Visakhapatnam, India. A voucher specimen (BSI/DRC/2021-22/Tech/434) was deposited at the Hyderabad Herbarium of the Botanical Survey of India. Sigma-grade streptozotocin (STZ) (500 mg) and glibenclamide were procured from Om Scientific Supplier, Berhampur, Odisha. The Institutional Animal Ethics Committee approved the animal study following CPCSEA guidelines (Approval Number: CUTM/IAEC-11/CPCSEA), New Delhi, India. All CPCSEA regulations and ethical standards were strictly followed throughout the experiment.

Selection of Extract for Further Herbal Ointment Formulations

This study aimed to develop novel herbal topical gel formulations incorporating *Plumeria pudica* leaf extract for effective wound management. *Plumeria pudica* leaf extract was obtained using the steam distillation method.[18] The bioactive extract was then incorporated into ointment formulations using excipients such as wool fat, hard paraffin, white soft paraffin, polyethylene glycol, sorbitol mono-oleate, liquid paraffin, Span-60, Tween-60, methyl hydroxybenzoate, and propyl hydroxybenzoate.[19] The excision wounds on the dorsal region of Wistar albino rats were topically treated with the newly formulated herbal ointments.[20] The ethanol extract of *Plumeria pudica* leaves exhibited the highest total phenolic and flavonoid content, indicating its potential for wound healing. [21]

Characterization of Herbal Ointment

Analysis of Herbal Ointment PH

The pH of the ointment was evaluated using a digital pH meter. The pH meter was calibrated using standard buffer solutions. A small amount of ointment was mixed with distilled water to create a slurry, and the pH was measured, and the reading recorded.[22]

Analysis of Herbal Ointment Spreadability

A parallel plate apparatus or a spreadability tester was used to measure the spreadability of the herbal ointment. About 500 mg of ointment was placed between two glass plates or slides. The force required to separate the plates or the distance the ointment spreads under a specific load was measured. Spreadability was calculated based on the measured parameters, such as the diameter of the spread or the force required.[23]

Determination of Herbal Ointment

A viscometer (Ostwald, model No. 171795, Japan) was chosen based on the ointment's viscosity range. The herbal ointment was prepared and allowed to equilibrate to the desired temperature. The ointment was placed in the viscometer, and the viscosity was measured at different shear rates or speeds. The herbal ointment viscosity was calculated based on the measured parameter as flow time. The measurement was repeated multiple times to ensure accuracy and reproducibility.[24]

Consistency or hardness of an ointment (Pharmacopeial Forum)

The samples were prepared by shredding or melting, ensuring they were carefully filled into containers without air bubbles and leveled to achieve smooth surfaces. They were then stored at 25 ± 0.5°C for 24 hours unless otherwise specified. Finally, the test sample was placed on a penetrometer base for analysis. [25] The procedure involved ensuring that the sample surface was perpendicular to the vertical axis of the penetrating object. The object was positioned at 25 ± 0.5°C, allowed to make contact with the sample, and then released for five seconds, after which the penetration depth was measured. This process was repeated for the remaining two containers to ensure consistency and accuracy.[26]

Tube Extrudability studies of ointment

A standard empirical test determined the force required to extrude the material from the tube. This method assessed shear behaviour in the rheogram region exhibiting plug flow, which occurs when the applied shear rate exceeds the material's yield value.[27] The method used in this study assessed the extrudability of the ointment formulation by measuring the percentage of ointment extruded from the tube upon applying finger pressure. A higher extruded quantity indicated greater extrudability, demonstrating improved ease of application. The formulation was filled into a sterile aluminium tube equipped with a 5 mm nozzle tip, and finger pressure was applied. The amount of ointment extruded was then measured to evaluate the tube's extrudability .[28]

Preliminary Phytochemical Screening

The ethanol extracts of *Plumeria pudica* leaves were subjected to qualitative chemical assays for phytochemical analysis. Various bioactive compounds, including alkaloids, phenolics, tannins, flavonoids, amino acids, carbohydrates, saponins, terpenoids, and anthraquinones, were identified using standard tests such as Mayer's, Wagner's, Ferric chloride ($FeCl_3$), Shinoda, Ninhydrin, Molisch, Foam, Keller-Killani, and Bornträger tests.[29]

Preparation of Plumeria pudica leaf Extract by Solvent Extraction

The plant parts were cleaned, cut into small pieces, dried, and ground into a fine powder. This powdered material was then subjected to ethanol extraction for one week to isolate the desired bioactive compounds.[30] The liquid extract was concentrated under reduced pressure using a rotary evaporator to obtain a dried extract. Subsequently, the coarse powder of *Plumeria pudica* leaves underwent continuous hot extraction with ethanol using a Soxhlet apparatus for 24 hours.[31] The solvents were then removed under pressure, and the extracts were concentrated under a vacuum at 40–60°C. The filtered extracts of *Plumeria pudica* leaves were evaporated under reduced pressure, dried in an oven to remove residual solvents, and stored in a cool, dry place for further study [32].

Quantitative Phytochemical Analysis of Plumeria pudica Leaf Extract

Total Phenol Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu phenol assay, a standard spectrophotometric method for quantifying phenolic compounds.[33] A 0.2 mg/mL stock solution was mixed with Folin-Ciocalteu reagent and 20% sodium carbonate. The mixture was then incubated in the dark for 30 minutes. Absorbance was measured at 765 nm using a spectrophotometer, and the experiment was conducted in triplicate to ensure accuracy and reproducibility. The calibration curve was generated using gallic acid as the standard reference compound.[34]

The extract's total phenolic content was calculated using the formula:

$$TPC = \frac{C x V}{M}$$

Where:
C = gallic acid concentration
V = extract volume
M = extract weight
The total phenol content was calculated as milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

Total Flavonoid Content (TFC)

The extract's total flavonoid content was calculated using the formula:

$$TFC = \frac{C x V}{M}$$

Where:
C = gallic acid concentration
V = extract volume
M = extract weight

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The calculation yielded the total phenol content in milligrams of gallic acid equivalent per gram of material (mg GAE/g) [35].

The solution was incubated for 30 minutes, and its absorbance was measured at 415 nm using a spectrophotometer.[36] Quercetin was used as a positive control, and the experiment was conducted in triplicate to minimize errors. Additionally, a calibration curve was constructed using quercetin, enabling the determination of the total flavonoid content in milligrams of quercetin equivalent per gram of the sample.[37] The following formula (Equation 2) was used to determine the quercetin equivalent in the plant sample:

Quercetin Equivalent (QE) was calculated using the formula:

$$QE = \frac{CxV}{M}$$

Where:
C = concentration of quercetin obtained from the calibration curve (mg/mL)
V = volume of the extract solution (mL)
M = weight of the extract (g)
The resulting data is given in Table 7**.**

GC-MS Analysis of Ethanol Extract of Plumeria pudica Leaves

GC-MS analysis was performed at CAIF (Guwahati Biotech Park) to identify the phytochemical constituents of the ethanol extract of *Plumeria pudica* leaves.

GC-MS Analysis of Ethanol Extract of Plumeria pudica Leaves
A Shimadzu GC-MS-QP2050 instrument fitted with a suitable column, HP-5MS(30 m x 0.25 mm x 0.25 μm), and a temperature program from 60°C to 280°C at 10°C/min. A small volume of 1.6 μL of the prepared sample is injected into the GC-MS instrument at a temperature of 250°C. Helium (1 mL/min) was used as Carrier Gas with Electron Ionization (EI)mode. The components of the extract are separated based on their boiling points and affinity for the stationary phase. The separated components are detected using a mass spectrometer, which provides a mass spectrum for each component. The resulting chromatogram and mass spectra were analyzed to identify the components of the extract.[38]

Preparation of Ointment base
The study assessed the organoleptic properties of 1.5% Carbopol 940 gels infused with 5% and 10% ethanol extracts of *Plumeria pudica* leaves. Various excipients, including wool fat, polyethylene glycol, and sorbitol monooleate, were incorporated into the formulation.[39] The topical herbal ointment composition is detailed in Table 2. The organoleptic properties of herbal gels containing *Plumeria pudica* extract were documented, showcasing their yellowish-green colour, smooth texture, and characteristic scent. Additionally, the ointment's pH, viscosity, spreadability, and tensile strength were assessed and compared to the gel base and povidone-iodine ointment, as presented in Table 2.

Chemicals and drugs
Sigma-grade streptozotocin (STZ) (500 mg), glibenclamide, and Nicotinamide were procured from OM Scientific Supplier, Berhampur, Odisha. The ointment base ingredients, including wool fat, cetyl alcohol, hard paraffin, white soft paraffin, polyethylene glycol-400, polyethylene glycol-4000, sorbitol monooleate, white beeswax, methyl hydroxybenzoate, and propyl hydroxybenzoate, were sourced from SCITECH SCIENTIFIC, 4/5 Mehta Building, 1st Floor, 260 Samuel Street, Vad Gadi, Masjid Bunder (W), Mumbai – 400003, India.

Experimental animals
The protocol previously reported was used in this experiment. Wistar albino rats of both sexes, weighing 150–200 g, were obtained from the Department of Pharmacology, Centurion University of Technology and Management, Bhubaneswar, Odisha, India. The rats were housed in polypropylene cages under controlled conditions, provided with standard pellets (Champaka Rat feed, Bangalore, India), free access to clean water, and adequate ventilation. Before the study, the animals underwent an acclimation period of two weeks to adapt to the laboratory environment.[40]
In the *in vivo* efficacy study, diabetes was induced in albino Wistar rats by administering streptozotocin (STZ) at 55 mg/kg following nicotinamide administration at 10 mg/kg. Diabetes was confirmed two days post-STZ administration using a fasting blood sugar (FBS) test.
The treatment protocol was carried out for 28 days, with the animals divided into the following groups:
Group I: Normal control
Group II: Induced control (STZ)
Group III: Treatment(STZ +*EEPP*) low dose
Group IV: Treatment (STZ +*EEPP*) medium dose
Group V: Treatment (STZ +*EEPP*) high dose
Biochemical parameters, including blood sugar levels, were assessed, and histopathological examinations of the pancreatic islets and wound tissue were conducted to evaluate the effects of the treatment.

In Vivo Wound Healing
Healthy albino Wistar rats of both sexes were selected for the study and acclimated to the laboratory environment before experimentation. A standardized excision wound was created on the animals' skin to ensure uniformity in the study.[41] The animals were divided into four groups, each consisting of five. The control group received water as a vehicle control, while the diabetic control group was administered streptozotocin (STZ) at 55 mg/kg body weight.[42] The test group received the ethanol extract of *Plumeria pudica* (*EEPP*) at 500 mg/kg body weight applied as an ointment to the wound area to evaluate its wound-healing efficacy. [43] The positive control group was treated with povidone-iodine ointment, a standard antiseptic, applied to the wound area for comparison with the test formulation.[44] The wound area was measured at regular intervals (every 2–3 days) using a digital camera or the wound tracing method to monitor the healing process.[45] The time required for complete wound healing was recorded, and the tensile strength of the healed tissues was measured. After the induction of diabetes (plasma glucose levels ≥ 250 mg/dL), a standardized full-thickness skin wound (2 mm × 5 mm) was surgically created on the dorsal surface of the right hindfoot of rats under ketamine-xylazine anesthesia.[46] Wound sizes were measured 24 hours post-wounding, after which treatment interventions were initiated. Histopathological examinations were conducted on both the wound tissue and the pancreatic islets. Additionally, *EEPP* was administered orally at 200 mg/kg body weight, and its effects were compared with the standard antidiabetic drug glibenclamide (5 mg/kg body weight). A vehicle control group was included, receiving distilled water, along with a diabetic control group in which rats were induced with STZ to evaluate the effects of the treatments in comparison to untreated and diabetic conditions. [47] Blood sugar levels were monitored at 7-day intervals over 28 days.

Measurement of closure of the wound (% of closure) with the Tensile strength.
Wound images were captured at intervals of days 0, 7, 14, and 21 using a digital camera with a scale in the frame to ensure accurate measurements. The wound area was analyzed using ImageJ software, which provided precise calculations of the wound size based on the captured photographs. This allowed for the determination of the extent of wound healing over time.[48] The following calculation was used to determine the percentage of wound closure:

$$\% \text{ Wound Closure} = \frac{Initial\ Wound\ Area - Current\ Wound\ Area}{Initial\ Wound\ Area} \times 100$$

On the twentieth day, wound tissue samples were collected from the rats for tensile strength testing. These tissue samples were carefully cut into homogeneous strips or dumbbell-shaped specimens, which were then attached to an Instron tensile strength testing instrument using suture material. The tissue samples were subjected to a controlled pulling force to quantify the tensile strength until they broke or separated. The testing parameters were standardized, including the maximum force applied and the test speed (10 mm/min). The mean tensile strength, percentage of wound closure, and standard deviation were calculated for each group to assess the healed tissue's healing process and mechanical strength. The data provided insight into the effectiveness of the treatment (e.g., *Plumeria pudica* extract) in promoting wound healing at the tissue level.

Histopathology of the Islets of Langerhans

The rats were euthanized, and the pancreas was carefully dissected and removed. The pancreas was fixed in a suitable fixative, 10% formalin, to preserve the tissue morphology. The fixed pancreas is processed for paraffin embedding, sectioning, and staining. Thin sections between 4-5 µm of the pancreas were cut using a microtome. The Sections were stained with Hematoxylin and Eosin (H&E) for general morphology. Sections are examined under a light microscope to evaluate islet morphology, cellular composition, and any pathological changes.[49]

Histopathology of the skin at different time scales

Rats were prepared for skin injury by wounding and topically applied the local anaesthesia lidocaine. Rats were euthanized at different time points for 24 hours to evaluate skin histopathology at various stages. Skin samples are collected from the treated area and any surrounding tissue. Skin samples are fixed in a suitable fixative, 10% formalin. The Sections were stained with Hematoxylin and Eosin (H&E) for general morphology. Sections are examined under a light microscope to evaluate islet morphology, cellular composition, and any pathological changes.[50]

Excision Wound Model

A previously reported model was used for this experiment.[51] The wound was carefully created on the dorsal side of albino Wistar rats using a scalpel, with a size of 1-2 cm in diameter. The wound extended through the epidermis, dermis, and hypodermis, ensuring that the damage affected all layers of the skin. While such a wound could heal naturally, therapeutic drugs are expected to accelerate the healing process. In this study, we applied standard drugs such as 5% povidone-iodine and EEPP (ethanol extract of *Plumeria pudica*) Ointment at 5% and 10% concentrations. The effects of these treatments on wound healing were evaluated and are presented in Table 5 and Figure 5. The data provided insights into how these treatments influenced the wound healing process, with a particular focus on the effectiveness of *EEPP* in promoting faster recovery compared to standard treatments.

Estimation of Blood Sugar Level

The albino Wistar rats were divided into seven groups, each comprising six individuals (n = 6–8). The groups included Standard Group (SG), Treated with glibenclamide at 5 mg/kg; Diseased Control (DC), STZ-induced diabetes group; Normal Control (NC), Treated with distilled water as vehicle control and *EEPP*-treated Groups: These groups were treated with different doses of *Plumeria pudica* ethanol extract (*EEPP*) at 100 mg/kg (*EEPP*-100), 200 mg/kg (*EEPP*-200), and 300 mg/kg (*EEPP*-300).To induce diabetes in the Diabetic Control (DC) group, streptozotocin (STZ) was administered intraperitoneally at a dose of 40–50 mg/kg. The Standard Group (SG) was treated with glibenclamide (5 mg/kg), and the Normal Control (NC) group received distilled water as the vehicle control. The *EEPP-treated* groups received different doses of the ethanol extract of *Plumeria pudica* (*EEPP*) in doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. Blood samples were collected from the tail vein of each rat on days 0, 7, 14, and 21. The blood glucose levels were measured using blood glucose test strips and a glucometer to monitor the effect of the treatments on glucose regulation.[52]

Results and Discussion

All chemical tests of the *Plumeria pudica* (*P. pudica*) leaf extract were positive for alkaloids, flavonoids, phenolic compounds, and terpenoids, which suggests that *P. pudica* possesses significant anti-inflammatory, antimicrobial, and antioxidant properties. These findings support the potential therapeutic applications of *Plumeria pudica* in treating various diseases. Further investigations are necessary to fully explore *Plumeria pudica's* pharmacological potential and develop it into effective natural remedies or drugs for various health conditions. Various chemical tests were conducted in the laboratory using standard reagents, including those for alkaloids, glycosides, phenols, flavonoids, and terpenoids. The results of the preliminary phytochemical screening of the test drug (*EEPP*) are presented in Table 1, and the total phenol and flavonoid content measurements for *EEPP* are shown in Table 7. These findings will guide further research on the therapeutic use of *Plumeria pudica* extract. A total of 78 compounds were identified through GC-MS analysis, of which 21 demonstrated significant therapeutic efficacy and pharmacological activity. The key compounds identified include 1-H Benzotriazole 4-Nitro, 1-Methyl-1-Ethyl-2 (Methoxycarbonylprop-2-yl)Hydrazine, 1,1,3,5,5-Hepta Methyl Tri siloxane, Thymol, 4-Tert Butyl Phenol, 2-Propanoic acid, and Strychnine. These compounds exhibit superior skin permeability, high absorption efficiency, and substantial therapeutic potential, offering anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. A comprehensive table listing all active ingredients isolated through GC-MS analysis is provided. These valuable compounds were derived from the leaf extracts of *Plumeria pudica*, as depicted in Figures 1 and 2 and summarized in Table 4. The physical examination of the *EEPP* ointment confirmed the presence of *Plumeria pudica* ethanol extract, with the ointment displaying a greenish-yellow color and a distinctive aroma. No phase separation was observed, and the ointment maintained a soft, semisolid consistency. The pH of the ointment was found to be 6.3, which is within the acceptable range for safe topical use. Additionally, the ointment exhibited a spreadability of 46.2 g/s and a hardness or consistency of 147 mm, as detailed in Table 3. Also, the blood sugar levels were measured in different groups of albino Wistar rats, including the normal control, diseased control (STZ-induced diabetes), standard group (glibenclamide, 5 mg/kg), and *EEPP*-treated groups (100 mg/kg, 200 mg/kg, and 300 mg/kg). When compared to the test drug *EEPP* at 300 mg/kg with the standard glibenclamide (5 mg/kg), the *EEPP* treatment demonstrated satisfactory efficacy in controlling blood sugar levels, as shown in the results presented in Table 6. The histopathological analysis revealed distinct findings across the experimental groups. The normal control group (A) exhibited intact pancreatic islet architecture, well-defined beta cells, preserved islet morphology, and no signs of inflammation or necrosis. In contrast, the diabetic control group (B) displayed significant pancreatic islet damage, characterized by a marked reduction in beta-cell mass, inflammatory cell infiltration, and necrosis, highlighting the detrimental effects of STZ-induced diabetes on pancreatic integrity. The positive control group (C), treated with glibenclamide, demonstrated partial restoration of pancreatic islet architecture, with moderate beta-cell regeneration and reduced inflammation, although not to the extent observed in the normal control group. Notably, the treated group (D), which received *Plumeria pudica* extract, showed substantial protection and restoration of pancreatic islets. This was evidenced by preserved beta-cell mass, reduced inflammation, and near-normal islet morphology, suggesting a significant regenerative and protective effect on pancreatic tissue. The STZ-induced diabetic control group exhibited extensive damage to pancreatic islets, while the positive control group (glibenclamide) showed moderate improvement in islet morphology. The *Plumeria pudica*-treated group displayed substantial protection and restoration of pancreatic islets, highlighting the potential therapeutic effects of *Plumeria pudica* extract. Histopathological findings suggest that the protective effects of *Plumeria pudica* extract on pancreatic islets may be attributed to its antioxidant, anti-inflammatory, and anti-diabetic properties. These results support using Plumeria pudica as a potential therapeutic agent for managing diabetes, as illustrated in Figure 3. The wound healing progression observed in the study revealed that by Day 10, the control group displayed minimal granulation tissue formation and collagen deposition, indicative of the initial phases of the healing process. In contrast, the diabetic control group demonstrated impaired wound healing, characterized by reduced granulation tissue formation and delayed collagen synthesis, consistent with diabetes-induced healing deficits. The positive control group, which received standard topical treatment, showed moderate granulation tissue formation and increased collagen deposition, suggesting enhanced healing. Notably, the *Plumeria pudica*-treated group displayed significant improvements, with enhanced granulation tissue formation, substantial

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collagen deposition, and early signs of epithelialization, indicating a faster and more efficient healing process compared to the diabetic control group.

Table 1: Result of preliminary phytochemical screening of ethanol extract of *Plumeria pudica*

Test	**Result**	**ethanol leaf extract (*EEPP*)**
Alkaloids		
Mayer's test	White flocculent precipitate	+
Wagner's test	Brownish-red precipitate	+
Hager's test	Yellowish sediment formed	+
Dragendorff's test	Orange Precipitates	-
Carbohydrates		
Molish's test	Violet ring	+
Benedict test	Orange precipitates	+
Fehling's test	Red Precipitates	-
Barfoed's test	Red Precipitates	-
Glycosides		
Keller-Killani test	Upper–red-brown, lower-blue green	-
Bornträger's test	Pink color	-
H2SO4 test	Violet-green color	+
Phenols/ tannins		
FeCl3test	Dark green color	+
Lead acetate test	White Precipitates	+
FolinCiocalteu test	Blue, green color	+
Flavonoids		
H2SO4 test	Yellow color disappear	+
Lead acetate test	Yellow Precipitates	+
Terpenoids		
Extract + copper acetate	Green Precipitates	+
Extract+chloroform+H2SO4	Forms a layer (red-brown color)	-
Steroids		
Salkowaski's test	Red color	-
Liebermann Burchard's test	Violet or Green color	+

By Day 14, the control group exhibited increased granulation tissue formation and moderate collagen deposition, reflecting continued healing progression. However, the diabetic control group maintained a pattern of delayed wound healing with persistently reduced collagen synthesis, highlighting the detrimental impact of diabetes on tissue repair. The positive control group demonstrated advanced granulation tissue formation and substantial collagen deposition, indicating significant progress toward wound closure. The *Plumeria pudica*-treated group showed marked epithelialization, increased collagen density, and near-complete wound closure, underscoring its superior healing potential relative to the diabetic control group. By Day 21, the control group achieved complete epithelialization and mature collagen deposition, signifying full wound healing. The diabetic control group, however, continued to exhibit delayed and incomplete wound healing, with limited collagen maturation and epithelialization. The positive control group displayed a fully healed wound with mature collagen, demonstrating the efficacy of standard treatment. Most notably, the *Plumeria pudica*-treated group exhibited complete wound closure, robust collagen remodelling, and restored skin architecture, highlighting its exceptional wound healing and tissue regeneration capabilities. The findings suggest that *Plumeria pudica* treatment significantly accelerates wound healing, enhances collagen deposition, and promotes tissue regeneration, with the treated group showing near-complete wound closure and restored skin architecture by day 21, as shown in Figure 4.The result of measurement of wound closure (% of closure) with the Tensile strength was also evaluated and calculated as the % of wound closure at 7-day intervals. The closure percentage rate of the test drug *EEPP* (ethanol extract of *Plumeria pudica*) was satisfactory, demonstrating a comparable effect to the standard drug povidone iodine ointment. The results of the wound closure analysis are summarized in Table 8. Additionally, the tensile strength of the wound tissue was calculated by dividing the maximum force applied to the wound by the cross-sectional area of the tissue. Statistical analysis revealed significant differences in tensile strength between the 5% and 10% *Plumeria pudica* leaf extract gel groups and the povidone-iodine ointment group at various time intervals ($p < 0.05$),

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suggesting that the *Plumeria pudica* extract promoted stronger tissue regeneration and collagen formation, which contributed to enhanced wound healing when compared to the standard treatment. This finding further supports the potential of *Plumeria pudica* as an effective therapeutic agent for improving wound healing, with its efficacy comparable to or exceeding that of the standard drug. At different time intervals, statistical analysis revealed significant differences in the percentage of wound closure between the test *EEPP* (ethanol extract of *Plumeria pudica*) group and

Table 2. Preparation of ethanol extract of *Plumeria pudica* (EEPP) ointment, the ointment bases, and their quantities

Ingredients	(Hydrocarbon Base) (g)	(Water soluble Base) (g)	(Water miscible Base) (g)	(Absorption Base) (g)
Wool fat	-	-	-	1.5
Hard paraffin	0.9	-	-	1.5
Ceto stearyl alcohol	1.5	-	4.5	1.5
White soft paraffin	27.0	-	-	25.0
Polyethylene glycol 4000	-	12	-	-
Polyethylene glycol 400	-	18	-	-
Sorbitol mono-oleate	-	-	-	-
Liquid paraffin	-	-	-	-
White beeswax	0.6	-	0.75	-
Span 60		-	2.25	-
Tween 60	-	-	1.5	-
Methyl hydroxyl benzoate	-	-	0.006	-
Propyl hydroxybenzoate	-	-	0.006	-
Purified water	-	-	21.0	
EEPP	1.0	1.0	1.0	1.0

Table 3: Physical Evaluation of Formulated (EEPP) ethanol extract of *Plumeria pudica* Ointment

Evaluation parameters	Herbal ointment
Colour	Greenish Yellow
Odor	Characteristic
Consistency	Soft semisolid
Phase separation	Nil
pH (10%w/v solution)	6.3
Hardness or consistency	147mm
Spreadability	46.2g/s

both the Control and Standard groups ($p < 0.05$). These results indicate that the *EEPP* formulation was more effective in promoting wound closure. The findings from the wound closure study demonstrated that the *EEPP* group closed wounds more rapidly, with the size of the wound decreasing significantly at each time point. Specifically, the *EEPP* group achieved 85.1% closure on day 2, 65.9% on day 4, and 44.6% on

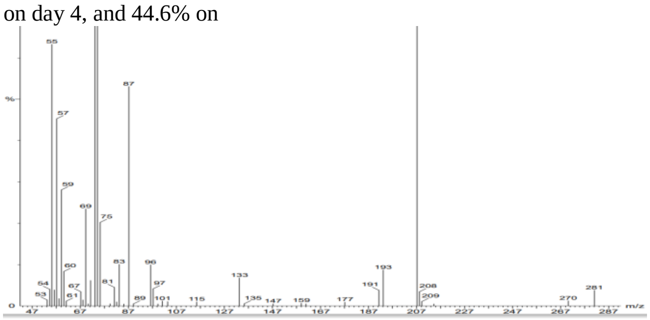

Figure 1. Mass-Spectroscopy Analysis of *Plumeria pudica* leaves *EEPP*.

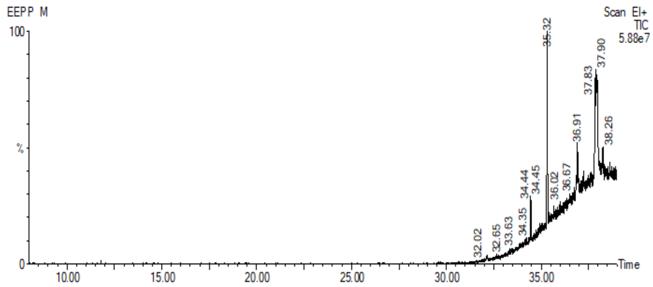

Figure 2: Gas Chromatography analysis of *Plumeria pudica* leaves EEPP

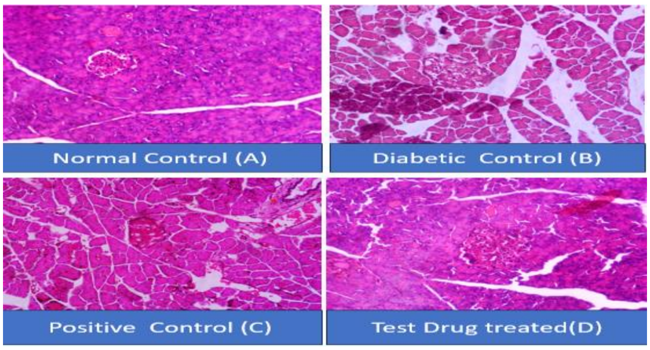

Figure 3: Histopathology of pancreatic islets from different groups of albino Wistars rats pancreatic Histopathology (21days study) like Normal control (A)(Received Water vehicle), Diabetic control (B)(Streptozotocin-induced diabetic model), Positive Control (C) (Receiving the Standard Drugs), and Treated group (D)(Receiving Test drug *EEPP*). 40x magnification

day 6. In contrast, the control and standard groups showed slower wound healing at similar time intervals, confirming the superior healing potential of the *EEPP* formulation.

This study indicates that applying a topical ointment containing an ethanol extract of *Plumeria pudica* leaves significantly accelerates wound healing. When compared to povidone-iodine ointment, which achieved 97.64% wound closure after 14 days, the ointments

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containing 5% and 10% ethanol extracts of *Plumeria pudica* resulted in 93.81% and 96.43% wound closure, respectively. By the twentieth day, histological analysis revealed that the 10% ethanol extract ointment showed the most pronounced improvement in wound healing, exhibiting normal skin structures. In contrast, the control group and the 5% extract group did not show the same level of healing. This suggests that the wound healing efficacy of the extract is dose-dependent, with higher concentrations of the extract (10%) yielding more effective

Table 4. GC-MS analysis of an extract of *Plumeria pudica* leaves with the phytochemicals and their absorption rate with skin permeability (cm/hr)

.

SL.NO	RT(min)	Name of the compound	CLASS	Molecular formula	Molecular weight	% of area Absorption	Skin permeability (cm/hr)
01	10.5-12.5	1-H Benzotriazole 4 Nitro	Heterocyclic	$C_6H_5N_3O_2$	151.13	5-15%	3.7-4.21
02	15.43	1-Methyl-1Ethyl-2(Methoxy carbonyl prop 2-yl)Hydrazine	Hydrazine	$C_9H_{18}N_2O_2$	203.14	10-25%	2.51-3.01
03	13.42-15.10	111,3,555-Hepta Methyl Tri siloxane	Siloxanes	$C_7H_{22}O_2Si_3$	236.55	5-15%	2.21-3.21
04	12.5-14.5	CycloTrisiloxane,Hexa Methyl	Siloxanes	$C_6H_{18}O_3Si_3$	222.48	2-8%	2.5-3.56
05	17.5-20.5	Hexestrol	Phenolic	$C_{18}H_{22}O_2$	270.42	15-30%	1.89-2.89
06	9.5-12.5	Thymol	Phenolic	$C_{10}H_{14}O$	150.24	25-40%	1.41-2.41
07	18.5-22.5	Strychnine	Alkaloids	$C_{21}H_{22}N_2O_2$	334.41	2-8%	3.21-4.21
08	12.5-15.5	4 Tert Butyl Phenol	Phenolic	$C_{10}H_{14}O$	134.22	10-25%	2.51-3.51
09	15.5-18.5	D-Mannitol	Polyol	$C_6H_{14}O^6$	182.17	5-10%	4.11-5.11
10	14.5-17.5	2Ethyl,4 Methoxy	Phenolic	$C_9H_{12}O_2$	152.19	15-30%	2.51-3.51
11	6.5-8.5	2 Propanoic acid	Carboxylic acid	$C_3H_4O_2$	72.06	30-50%	1.51-2.51
12	17.5-20.5	7 Tetra Decan -1-ol	Alkenols	$C_{14}H_{28}O$	212.38	10-25%	2.51-3.51
13	20-24	10-Penta Decan-01-ol	Alkenols	$C_{15}H_{30}O$	226.40	10-25%	2.51-3.51
14	12-15	Hepta Siloxane	Siloxanes	$C_7H_{18}SiO_3$	206.38	10-25%	2.51-3.51
15	15-18	Octa siloxane	Siloxanes	$C_8H_{24}SiO_4$	248.52	10-25%	2.51-3.51
16	10-12	Heptatatrien-1 One	Cyclohepta Trieonones	C_7H_6O	106.12	20-40%	1.51-2.51
17	18-22	Hexa Decanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	256.43	10-25%	2.51-3.51
18	12-15	Un Decanoic acid	Saturated fatty acid	$C_{11}H_{22}0_2$	186.29	20-40%	1.51-2.51
19	5-7	Butanoic acid	Saturated fatty acid	$C_4H_8O_2$	88.11	30-50%	1.51-2.51
20	14-17	Methyl-8 Methyl Nonanoate	Easter	$C_{11}H_{22}O_2$	186.29	10-25%	2.51-3.51
21	18-22	Tetra Decanoic acid	Saturated fatty acid	$C_{14}H_{28}O_2$	228.38	10-25%	2.51-3.51

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healing results.[53] The efficacy of *Plumeria pudica* leaf extracts in promoting wound healing was evaluated by comparing wound closure rates in incision wounds treated with various formulations. These included a control ointment base, povidone-iodine ointment, and two herbal ointments containing 5% and 10% ethanol extracts of *Plumeria pudica*.The tensile strength of the wound was assessed to measure the extent of wound healing.[54] This refers to the force required to split the healing skin, which indicates the strength and integrity of the newly formed tissue. Higher tensile strength typically correlates with improved wound healing and better tissue regeneration.

Table 5. The measurement of wound closure (% of closure) every 2 days of intervals from Day 0-20
.

Group	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20
C Control (Distilled water)	100%	89.2 ± 2.1	58.1 ± 4.2	41.5 ± 5.1	21.8 ± 6.3	12.9 ± 7.2	6.4 ± 8.1	3.3 ± 9.0	2.1 ± 9.5	1.1 ± 9.5	0.7 ± 9.8
Standard (Povidone iodine)	100%	79.5 ± 2.5	58.2 ± 3.8	36.8 ± 4.7	19.9 ± 5.5	10.2 ± 6.8	5.1 ± 7.9	2.4 ± 8.9	1.1 ± 9.6	0.6 ± 9.9	0.2 ± 10.1
Test (*EEPP)*	100%	85.1 ± 2.8	65.9 ± 3.8	44.6 ± 4.6	24.3 ± 5.7	11.9 ± 7.2	6.4 ± 8.4	3.1 ± 9.3	1.6 ± 9.8	0.8 ± 10.2	0.4 ± 10.5

(The p-values are less than 0.05, indicating a statistically significant difference between the four groups)

Table 6. The blood sugar level was tested every 7 days from Day 0-28.

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Control (STZ-induced diabetes)	85.2 ± 5.1	342.1 ± 15.6	351.9 ± 18.2	362.1 ± 20.1	369.2 ± 22.5
Vehicle Control (Distilled Water)	84.5 ± 5.3	339.2 ± 16.2	349.1 ± 19.1	359.2 ± 21.1	366.3 ± 23.2
Standard (Glibenclamide, 5mg/kg)	83.9 ± 4.9	221.9 ± 10.9	181.8 ± 12.9	141.9 ± 14.9	101.2 ± 16.9
EEPP-treated (200mg/kg)	83.5 ± 4.8	280.9 ± 12.8	240.8 ± 15.1	201.9 ± 17.3	162.1 ± 19.5 |

Table 7. The measurement of the test drug's total phenol and flavonoid content (*EEPP*).

Phytochemical Parameter	Method	Wavelength (nm)	Standard Used	Expression of Results	Results
Total Phenol Content	Folin-Ciocalteu	765	Gallic acid	mg GAE/g	124.56 ± 3.21
Total Flavonoid Content	Spectrophotometry	415	Quercetin	mg QE/g	56.78 ± 2.15

Table 8. Tensile Strength of Wounds at Different Time Intervals

Time Interval (days)	Povidone-Iodine Ointment (g)	5% *Plumeria pudica* Leaf Extract Gel (g)	10% *Plumeria pudica* Leaf Extract Gel (g)
0	0 ± 0	0 ± 0	0 ± 0
2	3 ± 0.9	4 ± 1.12	5 ± 1.3
4	4 ± 1.1	8 ± 1.23	11 ± 2.3
6	5 ± 1.3	11 ± 3.5	17 ± 3.7
8	7 ± 2.6	15 ± 4.1	21 ± 4.7
10	9 ± 1.8	19 ± 4.9	25 ± 5.1
12	11 ± 3.3	21 ± 5.2	27 ± 5.8
14	12 ± 3.6	23 ± 5.9	29 ± 6.1
16	12 ± 2.9	24 ± 6.1	30 ± 6.2
18	13 ± 3.17	25 ± 6.7	31 ± 6.8

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20	14 ± 3.15	24 ± 5.11	28 ± 4.05

(p-values are less than 0.05, indicating a statistically significant difference between the three groups)

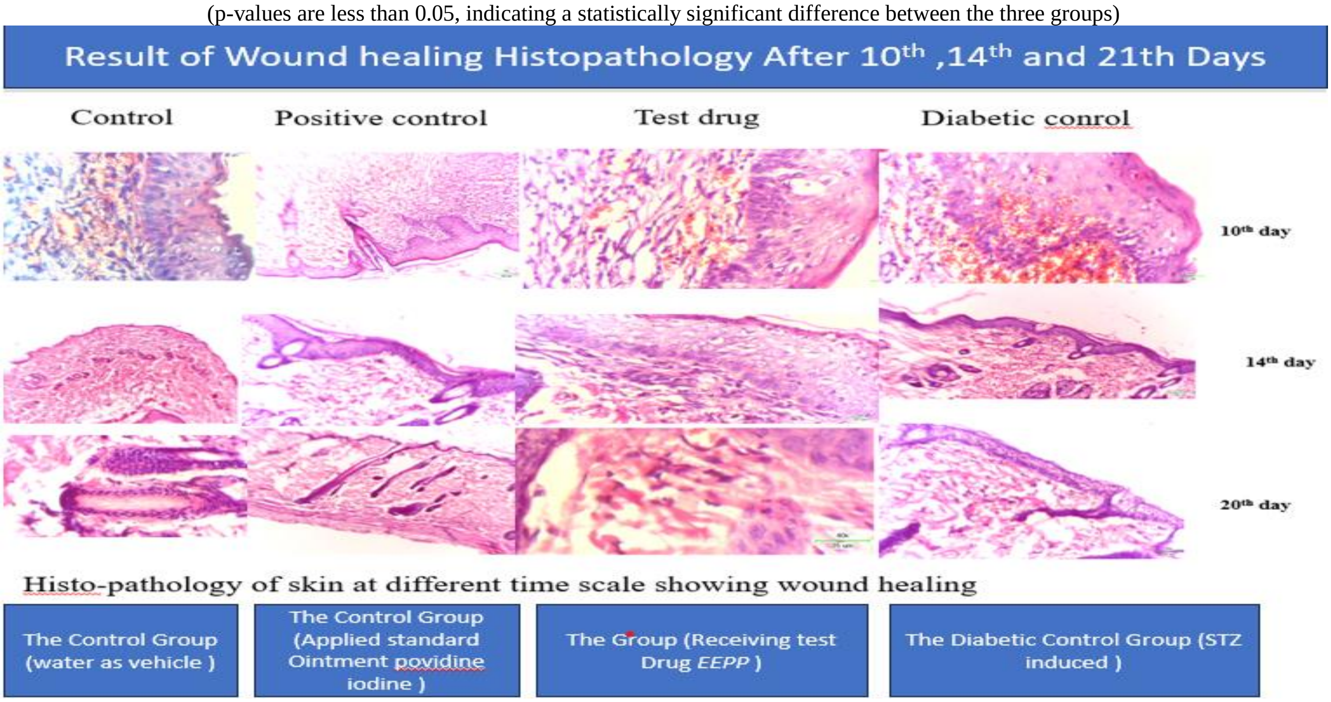

Figure 4: Histopathology of skin at different time scales showing wound healing result of 10th, 14th, and 20th days of the **(A)**Control Group,**(B)** Positive control Group, **(C)***EEPP* treated Test group, and **(D)** Diabetic control group**.** 40x magnification

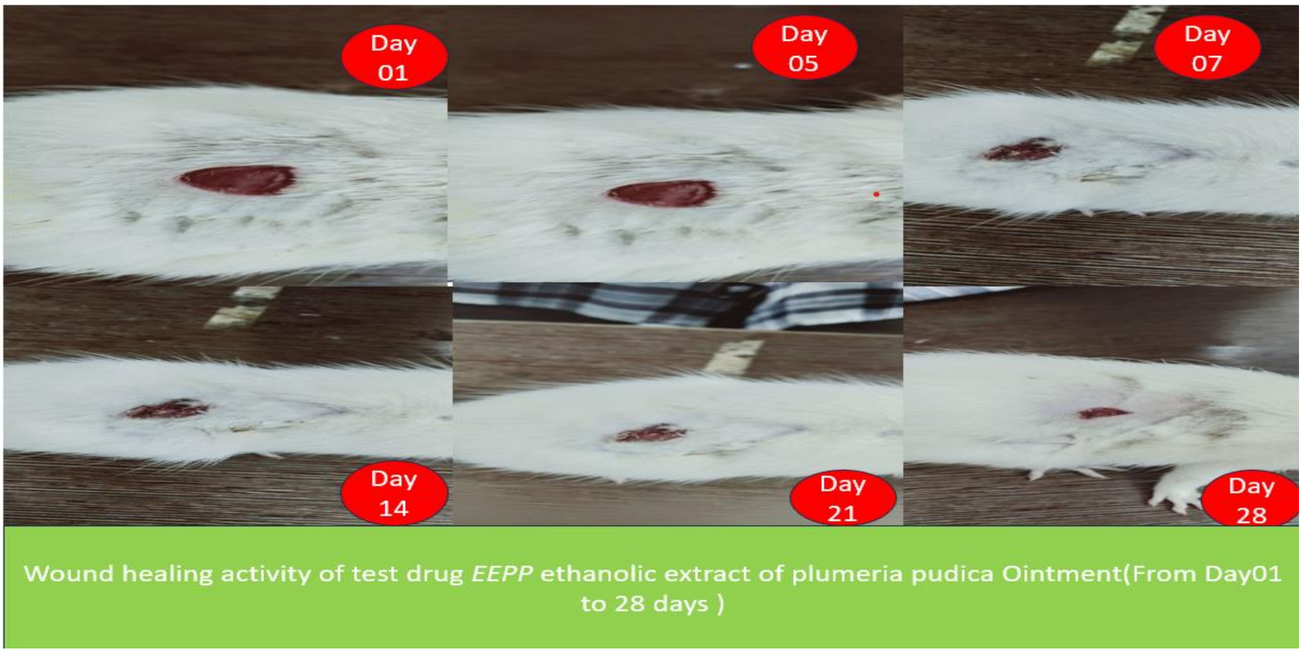

Figure 5: Wound healing effect of ethanol extract of *Plumeria pudica* (*EEPP*) ointment (Day 1-28 in 7-day intervals) 10x magnification normal wound

Wound healing treatments typically enhance tensile strength. On the twentieth day post-wounding, the rats treated with povidone-iodine ointment exhibited a tensile strength of 14 ± 3.15 g. In contrast, those treated with topical herbal gels containing 5% and 10% ethanol extracts of *Plumeria pudica* leaves displayed significantly higher tensile strengths of 24 ± 5.11 g and 28 ± 4.05 g, respectively (Table 7). The topical application of herbal gels containing *Plumeria pudica* leaf extract effectively promoted healthy granulation tissue formation in the wound area. Animals in the control group exhibited unhealthy granulation tissue, characterized by a dark red color, frequent bleeding upon touch, and signs of possible wound infection.[55] In contrast, the active ingredients of the *Plumeria pudica* extract appear to have enhanced skin penetration properties, promoting healthy granulation tissue formation and improved wound healing. The proteins platelet-derived growth factor (PDGF), c-myc, TGF-β, and β-catenin, crucial for wound healing, may interact with the active components in the *Plumeria pudica* leaf extract. This interaction potentially stabilizes the traditional signalling network, particularly the β-catenin pathway.[56] The primary function of this pathway is to regulate and enhance the

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healing process and control the growth and development of newly formed skin during the wound healing process. This mechanism, responsible for fibrosis and promoting regeneration, is activated in response to tissue damage.[57] When β-catenin is activated, it induces transcriptional changes that are crucial for both wound healing and the regulation of scar size. This process also plays a vital role in the proliferative phase of wound repair.[58] Additionally, studies have shown that non-healing wounds exhibit elevated levels of c-myc and β-catenin, which may contribute to the formation of excessive scarring.[59] Furthermore, several studies have shown that elevated TGF-β expression contributes to inflammation during the initial stages of wound formation.[60] The extract from *Plumeria pudica* leaves contains active compounds that effectively modulate protein activity and regulate the healing process. It is likely that the prolonged contact time, which may have enhanced skin permeability, played a significant role in the success of the topical herbal ointment derived from the ethanol extract of *Plumeria pudica* leaves. The present study aimed to explore the phytochemical and pharmacological properties of *Plumeria pudica* ethanol leaf extract and its polyherbal ointment formulation, specifically focusing on wound healing and anti-diabetic effects. Phytochemical screening of the ethanol leaf extract revealed the presence of several bioactive compounds, including flavonoids, phenolic acids, and alkaloids, which are known for their therapeutic potential.[61] these compounds are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties, crucial in promoting wound healing and exerting anti-diabetic effects, as observed in this study. The wound healing study results demonstrated that the polyherbal ointment formulation containing *Plumeria pudica* ethanol leaf extract significantly enhanced wound closure rates and tissue regeneration compared to the control group. This can be attributed to the synergistic effects of the bioactive compounds in the extract, which likely enhance collagen synthesis, improve tissue strength, and accelerate wound healing.[62] The anti-diabetic study demonstrated that the *Plumeria pudica* ethanol leaf extract exhibited significant glucose-lowering effects in diabetic rats, comparable to the effects of the standard anti-diabetic medication. These effects can be attributed to the bioactive compounds that may enhance insulin sensitivity, improve glucose uptake, and inhibit hepatic glucose production. The present study highlights the potential of *Plumeria pudica* ethanol leaf extract and its polyherbal ointment formulation in promoting wound healing and exerting anti-diabetic effects. Phytochemical analysis confirmed the presence of diverse bioactive compounds, likely responsible for the observed pharmacological benefits.[63] Further research is required to isolate and characterize the specific active constituents responsible for these therapeutic effects.

Conclusions

Topical herbal ointments formulated with *Plumeria pudica* leaf ethanol extract exhibited remarkable wound-healing efficacy in excision wounds in rats. The application of these ointments significantly improved histological markers and accelerated wound closure rates, highlighting their potential role in promoting tissue regeneration. These findings suggest that *Plumeria pudica* extract-based topical formulations may serve as effective therapeutic agents for wound management, offering a natural alternative for enhancing the healing process. Natural remedies, such as herbal extracts, present a safe and cost-effective alternative to conventional wound-healing treatments. These topical herbal formulations promote effective wound management and offer a sustainable solution, particularly in resource-limited settings. The development of a topical ointment incorporating *Plumeria pudica* leaf extract holds significant promise for addressing various skin conditions and complications associated with diabetes, potentially expanding its therapeutic applications beyond wound healing.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgment

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