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Research Article

Studies of the Physicochemical Properties, Stability, Irritability, and Efficacy of Red Boroco (*Celosia argentea*) Leaf Extract Patch for Diabetic Wound Healing

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Abstract

Red Boroco (*Celosia argentea*) leaves contain flavonoids that act as wound healers by promoting skin tissue repair and fibroblast growth. The 15% w/w *C. argentea* leaves extract was formulated into a patch for diabetic wound healing. This study aims to evaluate the physicochemical characteristics, physical stability, irritability, and efficacy of the *C. argentea* leaves extract patch for diabetic wound healing. *Celosia argentea* leaves were macerated in 70% ethanol, the flavonoid content was measured, and the extract was formulated into a patch. Evaluation of the patch included measurements of organoleptic properties, pH, weight uniformity, folding resistance, patch thickness, moisture content, and moisture uptake. A stability study was conducted using a freeze-thaw cycle method. The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) to confirm the irritability of the patch. The efficacy study was conducted by measuring the percentage of wound closure in male Wistar rats. The results showed that *C. argentea* leaves contained 1.101 ± 0.012 µg QE of flavonoids. The patch characteristics were as follows: pH 6.16 ± 0.01; weight uniformity 0.513 ± 0.038 g; folding resistance >300 folds; patch thickness 0.4 mm; moisture content 0.031 ± 0.028%; and moisture uptake 0.030 ± 0.009%. Statistical analysis (unpaired t-test) showed no significant changes in physical properties after the freeze–thaw cycle ($p > 0.05$), indicating good stability. The one-way ANOVA test of wound closure showed no significant difference between the patch group and the positive control group ($p = 0.948$). Patch exhibited good physical stability, no irritation, and an 89.88% wound-healing rate in the test animal.

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INTRODUCTION

Skin protects the human body from external threats, including ultraviolet (UV) radiation, bacteria, and harmful chemicals that can compromise health. Additionally, the skin plays a vital role in maintaining fluid balance and regulating body temperature[1]. Although skin possesses an intrinsic capacity to regenerate and repair damaged tissues under normal conditions[2], wound healing in diabetic patients is significantly prolonged due to impaired tissue repair mechanisms and secondary bacterial infections, often culminating in chronic wounds, potential amputation, and high mortality rates[3]. Consequently, developing alternative therapeutic strategies, such as herbal medicines incorporated into suitable drug-delivery carriers, such as transdermal patches, remains a critical research objective[4].

A patch is a drug-delivery system composed of a drug-loaded polymer matrix with a protective backing layer, designed for topical or transdermal application. Patches facilitate targeted drug delivery across various medical applications, including

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mitigating the severity of diabetic wounds[5]. In diabetic wound management, patch-based delivery can stimulate collagen synthesis and promote fibroblast proliferation, thereby accelerating re-epithelialization and enhancing tissue coverage while maintaining optimal wound hydration[6]. Herbal remedies have gained increasing attention due to their favorable safety profiles and minimal side effects. Among these, red Boroco (*Celosia argentea* L., red variant) exhibits notable wound-healing and antioxidant properties attributed to its rich content of secondary metabolites, such as flavonoids, tannins, and saponins[7]. Although previous research demonstrated that a 10% *C. argentea* leaf extract possesses significant wound-healing activity[8], advanced delivery systems for this extract remain under-investigated. Thus, a polymeric patch was selected in this study as an appropriate carrier for *C. argentea* leaf extract.

To ensure optimal performance, the patch must meet strict physicochemical specifications, including organoleptic properties, pH, weight uniformity, folding endurance, thickness, moisture content, and moisture uptake. In addition, stability testing is essential for evaluating product quality, safety, and efficacy over time[9,10]. Herein, a freeze-thaw cycling method was employed to assess thermal stability and prevent potential quality degradation, providing insight into formulation integrity under accelerated extreme storage conditions.

Furthermore, the potential for skin irritation and adverse reactions, such as inflammation, swelling, and redness, must be evaluated to ensure dermatological safety[11]. Initial irritation testing was conducted using the Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay on fertilized Leghorn eggs[12]. This alternative method accurately predicts irritancy potential without using live animals, adhering to the 3R principles of refinement, reduction, and replacement. To evaluate *in vivo* efficacy, a diabetic rat model was used to closely mimic human impaired-healing conditions and assess how the extract-loaded patch matrix promotes fibroblast proliferation, wound closure, and microenvironmental hydration[13]. This study is a preliminary investigation to evaluate the formulation potential of a *C. argentea* leaf extract patch, selected based on screening tests not reported here. Consequently, the objective of this research was to evaluate the physicochemical properties, stability, potential for skin irritation, and wound-healing efficacy of the formulated *C. argentea* leaf extract patch.

MATERIALS AND METHODS

Materials

Celosia argentea L. red variant leaves were freshly harvested and authenticated at the Biology Laboratory Instruction, Universitas Ahmad Dahlan, with the determination number 219/Lab.Bio/B/V/2024. Quercetin standard, aluminum chloride ($AlCl_3$), sodium acetate, dimethyl sulfoxide (DMSO), polyethylene glycol 400 (PEG 400), hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), alloxan monohydrate, and ketamine injection (50 mg/mL) were purchased from standard chemical suppliers. Ethanol (70%), sodium chloride (0.9% NaCl), sodium hydroxide (0.1 M NaOH), acetone, propylene glycol, and potassium chloride (KCl) were of analytical grade. Distilled water was used throughout the study. Commercial silver sulfadiazine 1% cream (10 mg/g, PT Darya-Varia) served as the positive control in animal studies. Equipment utilized included an oven, rotary vacuum evaporator (Maskot Mode RE 1100 V), water bath (Memmert), UV-Vis spectrophotometer (Shimadzu UV-1800), hotplate magnetic stirrer (Cimarec, Thermo Fisher Scientific), digital pH meter (OHAUS Starter 300), screw micrometer (Tricle, 0.01 mm accuracy), egg incubator (DMZ), and Whatman No. 40 filter paper. Biological media and models included fertilized Leghorn hen eggs for chorioallantoic membrane testing and male Wistar rats (130-200 g). GraphPad Prism 10.0.1 was used for statistical calculations.

Methods

Preparation of C. argentea leaves extract

Sorted *C. argentea* leaves were dried in an oven at 50°C for 8–10 hours and ground into a fine powder. Maceration was performed by adding 150 g of leaf powder to 750 mL of 70% ethanol and stirring continuously for 1 hour, followed by static immersion at room temperature for 24 hours. The resulting macerate was filtered through Whatman No. 40 filter paper using a Büchner funnel. The filtrate was concentrated using a rotary vacuum evaporator at 50°C coupled with a water bath to obtain a viscous extract.

Measurement of flavonoid content in C. argentea leaf extract

A standard stock solution of quercetin (100 ppm) was prepared in 70% ethanol and diluted to generate a calibration curve across concentrations of 4, 6, 8, 10, and 12 ppm. A 0.5 mL aliquot of each standard solution was mixed with 0.1 mL of 10% $AlCl_3$ solution, 0.1 mL of 1 M sodium acetate, 1.5 mL of 70% ethanol, and 2.8 mL of distilled water. The mixture was homogenized, and its absorbance was measured at 436.50 nm using a UV-Vis spectrophotometer[14]. For sample analysis, 10.0 mg of extract was dissolved in 10 mL of 70% ethanol in a volumetric flask. A 1 mL aliquot was combined with 0.1 mL of 10% $AlCl_3$ solution, 0.1 mL of 1 M sodium acetate, 1.5 mL of 70% ethanol, and brought to a final volume of 5 mL with distilled water. The absorbance was recorded at 436.50 nm using a cuvette, and total flavonoid content was expressed as quercetin equivalents (µg QE/mL).

Preparation of the C. argentea leaves extract patch

Based on preliminary screenings assessing physical characteristics and wound-closure efficiency (unpublished data), a single optimized formulation was selected for patch preparation, as detailed in **Table I**. Briefly, PVP and HPMC were hydrated in hot water in a warm mortar to form a uniform hydrogel (Mixture A). Mixture B, consisting of 70% ethanol, DMSO, and PEG 400, was prepared separately. *Celosia argentea* leaf extract and Mixture A were added to Mixture B under continuous stirring on a hotplate stirrer at speed setting 6 for 10 minutes. The final homogeneous mixture was left at room temperature for 24 hours to allow ethanol to evaporate, then stored in the dark.

Table I. Formulation design of *C. argentea* leaves extract patch.

Materials	Total (% w/w)	Functions
Celosia argentea leaf extract	15	Active substance
HPMC	3	Polymer
PVP	1	Polymer
PEG 400	5	Plasticizer
DMSO	1	Permeation enhancer
Distilled water	23	Solvent
70% Ethanol	ad 100	Solvent

Physicochemical properties evaluation of the C. argentea leaves extract patch

Organoleptic properties (specifically aroma, color, and texture) were evaluated visually and sensorially[15]. For pH determination, patches were soaked in 10 mL of distilled water (pH 7.0) for 2 hours, and measurements were recorded using a calibrated pH meter standardized against buffer solutions at pH 4.0, 7.0, and 10.0[16]. The target physiological pH range for transdermal patches is 4.0–8.0[17], although adjustments up to pH 6.0–8.0 are clinically relevant under skin wound conditions[18].

Folding endurance was determined by repeatedly folding the patch along the same line until rupture occurred; a folding count exceeding 300 folds without rupture indicated optimal mechanical flexibility[19]. Patch thickness was measured at four distinct locations using a screw micrometer, and the values were averaged[20]. Weight uniformity was evaluated by individually weighing 10 randomly selected patches per batch across three independent replicates; acceptable uniformity was defined as a coefficient of variation (CV) not exceeding 5%[9]. Moisture content was calculated by recording the patch's initial weight, then placing it in a desiccator for 24 hours to obtain the dry weight. Moisture content percentage was determined using **Equation 1**.

$$\text{Moisture content (\%)} = \frac{\text{Initial patch weight} - \text{Final dry weight}}{\text{Initial patch weight}} \times 100\% \qquad [1]$$

Moisture uptake was evaluated by conditioning dried patches in a desiccator containing a saturated KCl solution (84% ± 2% RH) at room temperature for 24 hours prior to reweighing[21]. Low moisture sorption indicates enhanced formulation stability and a reduced risk of microbial contamination[22]. Moisture uptake percentage was calculated using **Equation 2**.

$$\text{Moisture uptake (\%)} = \frac{\text{Post-exposure weight} - \text{Final dry weight}}{\text{Final dry weight}} \times 100\% \qquad [2]$$

Stability study of C. argentea leaves extract patch

Accelerated physical stability was evaluated using a freeze-thaw cycling protocol. Patches were subjected to freezing temperatures between 0°C and -25°C for 24 hours, followed by storage at room temperature (25°C- 30°C) for 24 hours, representing one complete cycle. The experiment was carried out over three consecutive cycles[23].

Irritation study of C. argentea leaves extract patch

The potential for skin irritation was evaluated *in vitro* using the HET-CAM assay. Fertilized Leghorn hen eggs were incubated in an egg incubator at 37 ± 0.5°C and 40% ± 4% RH for 3 days. Eggs were kept upright and rotated daily to ensure appropriate embryonic positioning and prevent membrane adhesion. On day 3, the eggshells were disinfected with 70% ethanol, carefully opened, and inspected to confirm embryonic viability and yolk integrity.

Only viable embryos exhibiting intact vascular networks were selected for testing. Test substances (0.2 mL of liquid or 0.2 g of semi-solid/solid sample) were applied directly onto the chorioallantoic membrane surface, and vascular changes were monitored continuously for 5 minutes[24]. Scoring and irritancy categorizations were assigned according to **Tables II** and **III**. The evaluation protocol included an untreated normal control group (CAM without intervention), a negative control (0.9% NaCl), three positive control groups (0.1 M NaOH for hemorrhage, acetone for hyperemia, and propylene glycol for coagulation), and the test formulation group.

Table II. Time-dependent vascular damage scoring system.

Irritation Phenomenon	Score at 0.5 min	Score at 2.0 min	Score at 5.0 min
Hyperemia	5	3	1
Hemorrhage	7	5	3
Blood coagulation	9	7	5

Table III. Cumulative irritation score classification.

Cumulative Score Index	Irritation Category Classification
0.0–0.9	Non-irritating
1.0–4.9	Weakly irritating
5.0–8.9	Moderately irritating
9.0–21.0	Strongly irritating

Efficacy study of C. argentea leaves extract patch

Ethical approval for animal studies was granted by the Research Ethics Committee of Universitas Ahmad Dahlan (Clearance No. 012406121). Eighteen male Wistar rats were housed individually and acclimatized for 7 days under standard environmental conditions with *ad libitum* access to food and water. Animals were randomly allocated into three experimental groups (n = 6 per group): positive control, negative control, and treatment group. On day 8, baseline blood glucose levels were recorded, and diabetes was induced via an intraperitoneal injection of alloxan monohydrate (150 mg/kg BW). Blood glucose concentrations were measured on day 3 post-induction; rats with blood glucose levels > 200 mg/dL were confirmed to be diabetic and included in the study. Anesthesia was induced via intramuscular injection of ketamine at a dose of 0.2-0.3 mL. Upon complete anesthesia, the dorsal region was shaved, disinfected, and a full-thickness circular excision wound (22 mm diameter, 2 mm depth) was created[25].

Treatment was administered daily for 21 days. The positive control group received commercial silver sulfadiazine cream, the negative control group received a placebo patch lacking extract, and the treatment group received the 15% *C. argentea* leaf extract patch. Therapeutic efficacy was evaluated by assessing wound exudation/drying rate, wound diameter reduction, and calculating the wound healing percentage using **Equation 3**.

$$\text{Wound healing (\%)} = \frac{\text{Initial wound diameter on day 0} - \text{Residual wound diameter measured on designated observation days}}{\text{Initial wound diameter on day 0}} \times 100\% \quad [3]$$

Data analysis

Statistical analyses were performed using GraphPad Prism software. Data normality was assessed using the Shapiro-Wilk test, with a p-value > 0.05 indicating normality. Pre- and post-stability physicochemical properties following freeze-thaw cycles were compared using an unpaired Student's t-test to evaluate the significance of formulation changes; differences

were considered non-significant at $p > 0.05$, confirming formulation stability. Differences in wound closure percentages among the treatment groups were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparisons test to assess pairwise group differences. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Extraction of *C. argentea* leaves using 70% ethanol yielded 79.56 g of dense extract, corresponding to a yield value of 19.89%. Extract yield reflects the total quantity of active constituents extracted, where higher values indicate greater recovery of bioactive components. The obtained yield meets the benchmark for high-efficiency extraction, exceeding the established standard threshold of >10%14. Total flavonoid content was determined spectrophotometrically via $AlCl_3$ complexation, which produces a yellow complex detectable by UV-Vis spectrophotometry[26]. Quantification was performed using a quercetin standard curve, yielding a linear regression equation of y = 0.0552x - 0.0784 (**Figure 1**) and an extract flavonoid concentration of 1.101 ± 0.012 µg QE/mL. Polyphenolic flavonoids represent the primary class of secondary metabolites in *C. argentea*, with red plant varieties characteristically accumulating elevated levels of flavonoid-derived pigments, including anthocyanins and carotenoids.

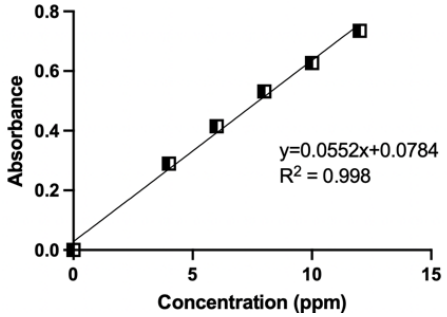


Figure 1. Linear regression curve of quercetin standard solution.

The target physical and chemical attributes of the formulation are outlined in **Table IV**. Organoleptic evaluations were conducted before and after a freeze-thaw stability cycle to assess changes in color, odor, and texture using visual and tactile inspection. Formulations that maintain consistent organoleptic features after stability testing are considered stable. Visual examination of the patch (**Figure 2**) revealed no discernible alterations in color, aroma, or texture following thermal stress. The patch retained its initial greenish-brown color, a characteristic attributed to the extract phytoconstituents, as previously described by Ranjan and Deokule[27], alongside its aromatic odor, smooth surface texture, and desirable elasticity. These observations confirm that the patch maintains its organoleptic integrity under extreme environmental conditions.

Table IV. Target of physical and chemical quality attributes of the patch.

Physical and chemical properties	Specification
pH	4 – 8[17]
Patch folding resistance	>300 fold[20]
Patch thickness (mm)	<1[9]
Weight uniformity (%)	CV values <5%[9]
Moisture content (%)	<10[17]
Moisture uptake (%)	<10[22]

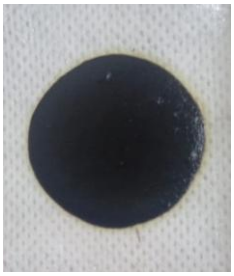


Figure 2. Image of *C. argentea* leaves extract patch.

Wound healing is a complex biological cascade in which local pH is a key physiological indicator of tissue recovery, infection risk, and cellular activity. Polymeric patches serve as protective wound dressings engineered to optimize tissue repair[28]. While healthy human epidermis has a slightly acidic surface pH (4.5–5.3) that increases to ~6.8 in the deeper stratum corneum, disruption of skin integrity exposes underlying tissues at physiological pH (~7.4), shifting the wound bed toward an alkaline state. Consequently, topical dressings must maintain a pH compatible with physiological tissue parameters (6.0–8.0) to promote healing[18].

As depicted in **Figure 3**, the initial average pH of the patch was 6.31, which decreased slightly to 6.16 following freeze-thaw testing. Minor pH reductions during thermal cycling are typically associated with slight polymer breakdown or the intrinsic acidity of formulation excipients, such as HPMC (pH 5.0–8.0) and PVP (pH 3.0–7.0)[29]. An unpaired t-test revealed no statistically significant difference in pH before and after cycling ($p = 0.0552$, $p > 0.05$), confirming the matrix's chemical stability.

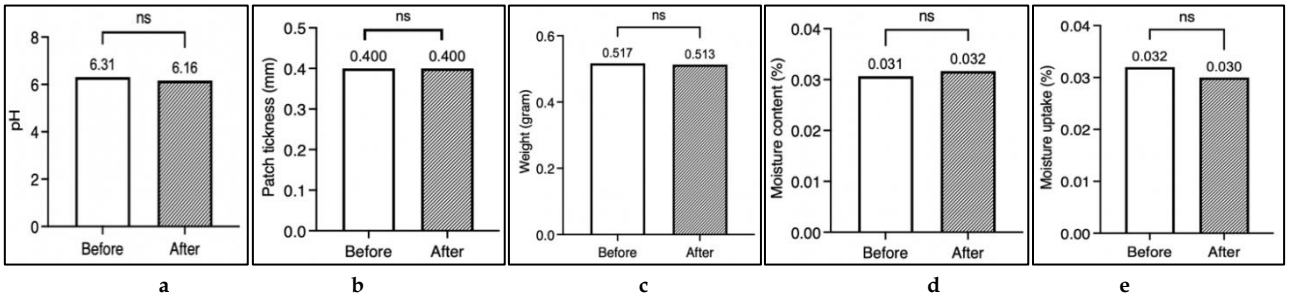


Figure 3. The results of the physicochemical properties of the patch before and after the freeze-thaw cycle on (**a**) pH, n=3; (**b**) patch thickness, n=3; (**c**) weight, n = 10; (**d**) moisture content, n=3; (**e**) moisture uptake, n=3.

Mechanical flexibility and structural integrity are critical parameters for patch utility. Folding resistance is governed by the synergistic interaction between polymers and plasticizers, with values exceeding 300 folds without tearing, thereby defining suitable mechanical durability[17]. The *C. argentea* patch maintained a folding resistance >300 folds both initially and after three freeze-thaw cycles (**Figure 4**), demonstrating resilience under variable storage temperatures. This structural endurance is driven by HPMC and PVP acting synergistically to form a flexible, non-brittle matrix, augmented by the plasticizing action of PEG 400[30]. Furthermore, the hydrophilic nature of PVP aids water retention, maintaining a flexible gel phase that stabilizes mechanical properties over time[9].

Figure 4. Image of *C. argentea* leaves extract patch before (**a**) and after (**b**) fold resistance measurement.

Uniform patch thickness is crucial for ensuring homogeneous drug distribution, consistent active constituent release profiles, and reliable skin permeation. Excessive thickness creates prolonged diffusion pathways that impede constituent release, whereas optimized thickness promotes efficient active delivery[22]. The baseline patch thickness was 0.40 mm and remained unchanged after freeze-thaw stress. A one-sample t-test yielded a standard deviation (SD) of 0, confirming that thermal stress did not alter patch dimensions and highlighting the physical resilience of the polymer network. Weight uniformity testing evaluates matrix homogeneity and dosing consistency across individual patches[9]. Minimal weight variation (small SD) indicates uniform batch manufacturing. The initial patch weight was 0.517 ± 0.027 g, which shifted marginally to 0.513 ± 0.038 g post-cycling. Statistical analysis using an unpaired t-test showed no significant weight loss ($p = 0.7916$, $p > 0.05$), confirming batch stability.

Moisture content directly governs formulation stability and microbial vulnerability, as elevated water levels promote bacterial and fungal proliferation[17]. Initial moisture content was recorded at 0.031 ± 0.027% and remained virtually identical at 0.031 ± 0.028% after freeze-thaw testing, well below the regulatory maximum of 10%. Unpaired t-test analysis confirmed no significant variation ($p = 0.9783$, $p > 0.05$). Similarly, moisture uptake evaluations assess environmental moisture absorption, which could otherwise compromise patch safety and structural performance[17]. Initial and post-cycling moisture uptake values were 0.032 ± 0.011% and 0.030 ± 0.009%, respectively. The low moisture uptake is attributed to the moderate hydrophilicity of the HPMC/PVP polymer blend under ambient conditions. Statistical comparison showed no significant change ($p = 0.8268$, $p > 0.05$), confirming overall environmental stability. Collectively, physical characterization and freeze-thaw cycling studies demonstrate that the *C. argentea* extract patch maintains robust physical stability. Extreme thermal changes did not compromise its organoleptic properties, pH, folding resistance, thickness, weight uniformity, water content, or moisture uptake, with all parameters consistently fulfilling regulatory specifications.

Vascular irritation and inflammatory potential were evaluated using the HET-CAM assay on fertilized Leghorn chicken eggs. The experimental setup included a neutral reference group, a negative control (0.9% NaCl) to establish baseline physiological compatibility, and positive controls representing classical irritation responses: 0.1 M NaOH (hemorrhage), acetone (hyperemia), and propylene glycol (coagulation). Treatment groups comprised patch formulations before and after freeze-thaw testing (**Tables V** and **VI**). Application of 0.1 M NaOH induced severe vascular rupture (hemorrhage) persisting through 5 minutes, yielding a strong irritation score of 20.333 ± 1.150. Acetone induced rapid tissue erythema and vasodilation (hyperemia) within 1 minute, resulting in a cumulative score of 16.333 ± 1.154. Propylene glycol caused intravascular protein precipitation (coagulation), with a score of 9.333 ± 1.154[24]. In contrast, patch preparations, both before and after freeze-thaw testing, produced a zero irritation score (0 ± 0), with no signs of hemorrhage, hyperemia, or coagulation. The complete absence of vascular reactions, matching the negative control, confirms that the formulation is non-irritating and bio-compatible for topical application on wound sites.

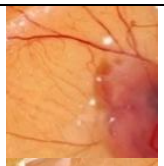
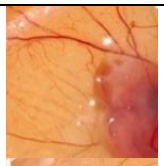
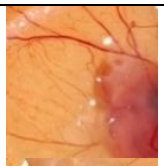
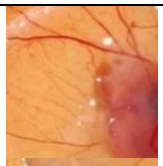
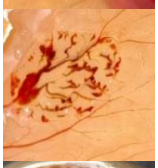
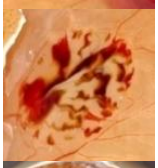
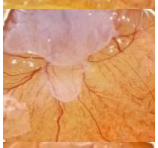
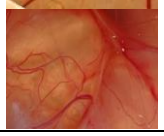
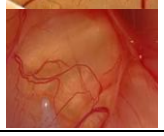
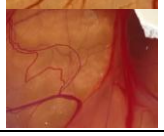
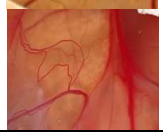
Table V. HET-CAM data interpretation results.

Test Solution	Cumulative Score	Interpretation
Neutral	0 ± 0	Non-Irritating
NaOH 0.1M	20.333 ± 1.150	Strong
Acetone	16.333 ± 1.154	Strong
Propylene Glycol	9.333 ± 1.154	Strong
NaCl 0.9%	0 ± 0	Non-Irritating
Before the cycling test	0 ± 0	Non-irritating
After the cycling test	0 ± 0	Non-Irritating

Due to the absence of commercial herbal patches for burn and wound care, silver sulfadiazine cream, an established clinical standard for topical burn management, was selected as the reference control[31-33]. Wound repair progresses through distinct, overlapping phases: hemostasis/inflammation, proliferation, and tissue remodeling[34]. The maturation phase spans from 21 days to 2 years, requiring balanced cell apoptosis and extracellular matrix deposition[35]. In diabetic conditions, wound healing is markedly impaired by persistent inflammation, deficient angiogenesis, and compromised cellular proliferation, often delaying recovery beyond the typical 2–3-week period seen in healthy tissue[36,37]. Consequently, a 21-day observation window was chosen to encompass the inflammatory and proliferative stages while capturing the onset of tissue remodeling. Throughout the 21-day evaluation, test rats maintained blood glucose levels above 200 mg/dL (**Figure 5**), confirming sustained hyperglycemia. Macroscopic observation revealed initial wound closure by day 3 post-treatment (**Figure 6**), marked by scab formation and epidermal thickening. By day 21, wound closure reached 85% (**Figure 7**).

Data normality was confirmed using the Shapiro-Wilk test ($p > 0.05$). One-way ANOVA revealed significant differences in wound closure rates among treatment groups ($p = 0.003$). Post-hoc multiple comparison testing demonstrated no statistically significant difference between the positive control (silver sulfadiazine cream) and the 15% *C. argentea* patch group ($p = 0.948$), whereas the negative control (patch base) exhibited significantly lower closure rates. The equivalent efficacy of the 15% *C. argentea* extract patch to that of commercial silver sulfadiazine cream highlights the formulation's therapeutic potential for managing diabetic wounds.

Table VI. Irritation reaction on the CAM surface.

Treatment	0 sec	30 sec	2 sec	5 sec
Neutral				
NaOH 0.1M (Hemorrhage Control)				
Acetone (Hyperemia Control)				
Propylene glycol (Coagulation Control)				
NaCl 0.9%				
Before the freeze-thaw cycle				
After the freeze-thaw cycle				

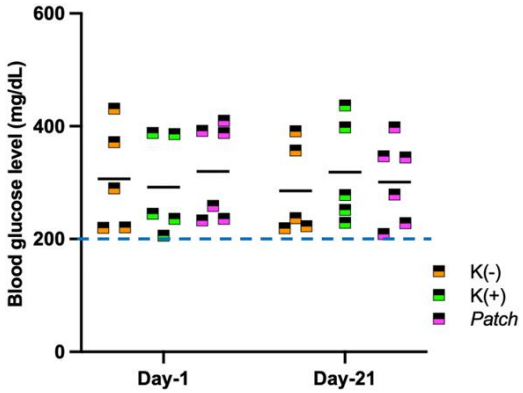


Figure 5. Blood glucose levels in rats were monitored during the wound-closure study from day 1 to day 21.

Hydrogel patch delivery systems offer distinct advantages over conventional ointments or creams by maintaining local wound moisture, facilitating cell migration, and preventing rapid evaporative water loss[38]. In this formulation, HPMC forms a hydrophilic, three-dimensional polymer network that absorbs exudate while maintaining a moist microenvironment essential for rapid re-epithelialization. Furthermore, the HPMC/PVP matrix functions as a controlled-release system, enabling sustained delivery of bioactive phytochemicals over prolonged periods, a feature particularly beneficial for chronic, slow-healing diabetic ulcers[39,40]. The inclusion of PVP enhances pressure-sensitive adhesion, ensuring firm contact with the wound bed, protecting the tissue from external pathogens, and maintaining localized active concentration[41].

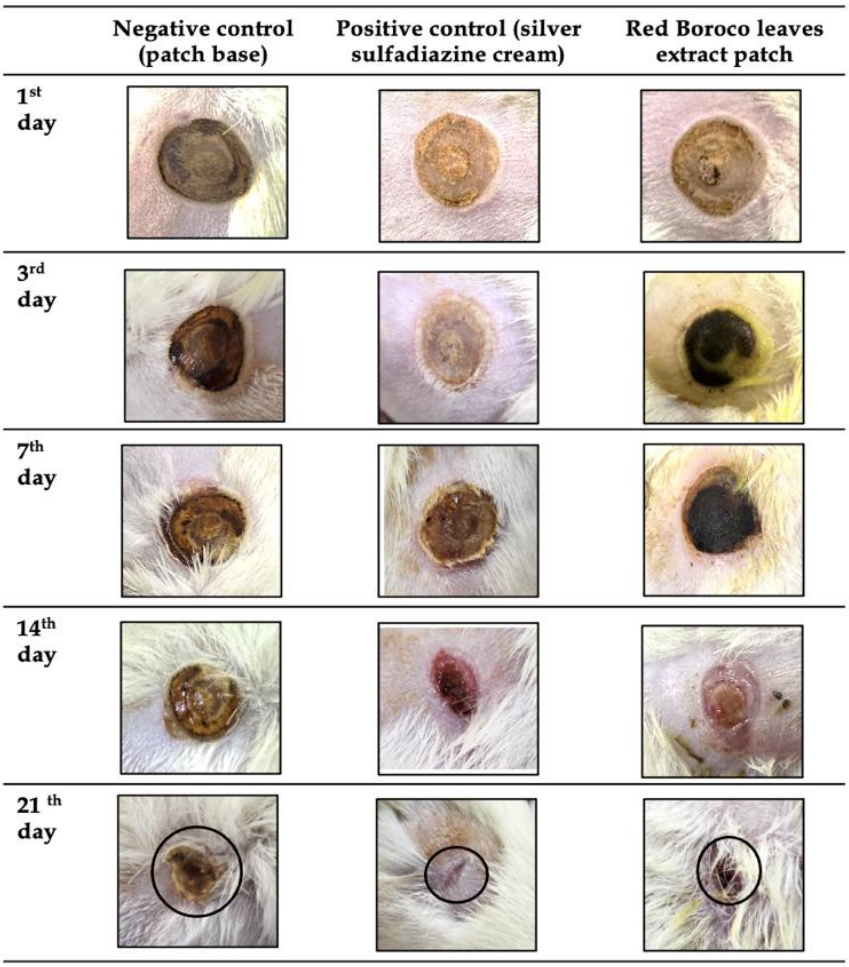


Figure 6. Efficacy Study of *C. argentea* Leaves Extract Patch.

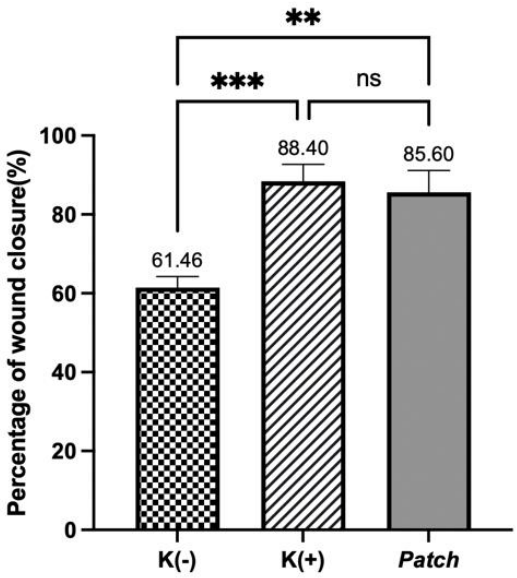


Figure 7. Wound closure percentage in rats.

The therapeutic action of the patch is primarily mediated by plant flavonoids present in the extract, which play multifaceted roles in tissue regeneration[42]. Flavonoid-loaded hydrogels have been shown to accelerate wound repair by suppressing oxidative stress and inflammatory cascades while stimulating proliferation and tissue remodeling[43]. Mechanistically, flavonoids inhibit the NF-κB pathway, downregulating pro-inflammatory cytokines (TNF-α, IL-1, IL-6), while concurrently

activating the Nrf2/ARE pathway to scavenge reactive oxygen species (ROS)42. Furthermore, flavonoids promote cell migration, proliferation, and angiogenesis via the MAPK/ERK and PI3K/Akt pathways, drive extracellular matrix deposition through TGF-β signaling, and exert anti-apoptotic effects by modulating the JNK and p38 MAPK cascades[44].
This study was limited to a single optimized formulation derived from preliminary screening data. Additional limitations include the absence of comprehensive extract standardization parameters, such as loss on drying, moisture content, total ash content, and full phytochemical profiling. Given that hydroethanolic extracts retain moisture and are susceptible to batch variation, rigorous quality-control standardization remains essential to ensure batch reproducibility and chemical stability[45]. Future investigations should incorporate thorough extraction standardization and detailed molecular assays to further validate the formulation's robustness. Nevertheless, the physical stability, non-irritating nature, and comparable therapeutic efficacy to standard clinical care demonstrate that the 15% *C. argentea* extract patch holds significant promise as an advanced topical delivery system for diabetic wound management.

CONCLUSION

The transdermal patch formulation incorporating 15% *C. argentea* leaf extract demonstrated optimal physicochemical characteristics and high physical stability during accelerated freeze-thaw evaluations, maintaining structural integrity in organoleptic parameters, surface pH, film thickness, weight uniformity, folding endurance, moisture content, and moisture uptake capacity. Safety screening using the HET-CAM bioassay confirmed that the 15% extract patch induced no microvascular irritation, hyperemia, hemorrhage, or intravascular coagulation in the chorioallantoic membrane, thereby verifying its non-irritating nature and biocompatibility for dermal application. Furthermore, *in vivo* biological evaluations in diabetic Wistar rat models demonstrated that the 15% *C. argentea* leaf extract patch significantly accelerated full-thickness burn wound healing and re-epithelialization, achieving therapeutic closure efficacy comparable to the standard positive control, commercial silver sulfadiazine cream.

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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