**Ind. J. Pharm. Edu. Res., 2026; IPC Issue:15-27.**
https://www.ijper.org

Original Article

# Development of Topical Gel Containing Natural Extracts and Nano Silver for Wound Healing

**Arpitha, Poojitha Minakanagurki Ghanashyam Babu, Deveswaran Rajamanickam***

Department of Pharmaceutics, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, INDIA.

## ABSTRACT

**Background/Objectives:** The work undertaken focuses on the development of a topical gel containing natural extracts and nano silver for wound healing, using *Andrographis paniculata* and *Punica granatum* as sources of tannins and flavonoids, respectively. **Materials and Methods:** The plants were authenticated and extracted using microwave-assisted method of extraction. In order to confirm the presence of tannins, Ethanol extracts of *Andrographis paniculata* and *Punica granatum* leaves underwent phytochemical screening. These extracts were then characterized through GC-MS, followed by *in silico* studies against selected proteins involved in wound healing. Xanthan gum and Carbopol 940 was used as a gelling agent to create topical gel formulations. Percentage yield, spreadability, pH, viscosity, and *in vitro* permeation studies were used to assess the gel formulations. *In vivo* wound healing studies were conducted, assessing parameters such as Complete Blood Count (CBC), lipid profile test, wound contraction rate, and histopathological changes. **Results:** Among the six formulations prepared, formulation F2 was preferred based on its viscosity, pH, spreadability, and drug content. The binding affinities of the selected ligands to the chosen proteins were analysed, and it was concluded that ligands such as 24-Norursa-3,12-diene, anthraquinone, 1-(o-chlorophenyl), 1-butanol, 3-methoxy-, Indoline, 2-(hydroxydiphenylmethyl)-,tetrahydro-22-desoxy-tomatillidine, 3-hydroxy-6-methoxyflavone, and anthraquinone, 1-(o-chlorophenyl)- exhibited better binding affinities and maximum docking scores compared to other ligands. When compared to a positive control group the developed formulations showed notable wound healing in an excision wound model. **Conclusion:** In conclusion, the outcomes of the investigations showed that the topical gel formulations made with plant extracts had efficient wound healing activities.

**Keywords:** *Andrographis paniculata*, Herbal formulation, Nano silver Particles, *Punica granatum*, Wound healing.

**Correspondence:**
**Dr. Deveswaran Rajamanickam**
Department of Pharmaceutics, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, INDIA.
Email: deveswaran.ps.ph@msruas.ac.in

## INTRODUCTION

Topical drug administration is a localized drug method of delivery that can be administered via the skin, rectal, vaginal, or ocular channels, among other places on the body. These products have developed from basic ointments to complex delivery systems and they are primarily employed to treat skin disorders. This improvement has been greatly aided by a thorough understanding of drug-product-skin interactions. The initial step in topical drug delivery involves the drug permeating through the stratum corneum to reach the deeper layers of the epidermis and dermis. Notably, the drug does not accumulate in the dermal layer. Various forms of topical medications exist, including lotions, gels, patches, and powders, but they are most commonly formulated as creams, gels or ointments. The formulations are created within a vehicle or base, which can be customized for specific body sites or skin conditions. Pharmaceutical gels are semisolid formulations that contain a large amount of liquid trapped within a network of colloidal particles. These gels can be clear or opaque and are made using various substances, including natural gums, semi-synthetic materials, synthetic polymers, and clays. They have a jelly-like consistency due to a gelling agent and are used for their ease of application and ability to deliver active ingredients effectively.[1] Based on the manner in which the solvent and polymer interact, gels are broadly classified into organogels and hydrogels. Organogels consist predominantly of non-polar components and can incorporate limited amounts of water, enabling the delivery of hydrophilic drugs with tunable release profiles. In contrast, hydrogels are highly hydrated polymeric networks (up to 90% water) capable of swelling and mimicking biological tissues, making them particularly suitable for wound healing applications.[2] Wound is defined as a disruption in the structural and functional integrity of the skin and underlying tissues. Effective wound management remains a significant clinical challenge, especially in chronic conditions where healing

**DOI:** 10.5530/ijper.ipc003

**Copyright Information :**
Copyright Author (s) 2026 Distributed under Creative Commons CC-BY 4.0

**Publishing Partner :** Manuscript Technomedia. [www.mstechnomedia.com]

is impaired.[3] In order to restore tissue integrity, hemostasis, Inflammation, proliferation and remodeling must all take place in unison throughout the intricate, multi-phase process of wound healing.[4,5] However, factors such as infection, oxygenation, age, diabetes and nutrition can delay or impair this process, necessitating the development of advanced therapeutic strategies.

In recent years, herbal medicines and phytoconstituents have gained considerable attention due to their antimicrobial, anti-inflammatory, antioxidant, and tissue regenerative properties. Compounds derived from plants have been shown to enhance key healing mechanisms, including angiogenesis, collagen deposition, and re-epithelialization. Medicinal plants such as *Punica granatum* and *Andrographis paniculata* have been well investigated for their wound healing potential due to their rich phytochemical composition.[6] *Punica granatum* contains bioactive compounds like punicalagins, anthocyanins, and ellagic acid, which exhibit strong antioxidant, anti-inflammatory, and antimicrobial activities, thereby reducing oxidative stress, preventing infection, and enhancing collagen synthesis.[7] Similarly, *Andrographis paniculata*, rich in diterpenoids such as andrographolide, demonstrates significant anti-inflammatory, antimicrobial, and antioxidant properties that support tissue regeneration and accelerate wound closure.[8,9] These plants have been incorporated into various traditional and marketed herbal formulations, highlighting their therapeutic relevance in wound management. However, their legitimate clinical potential is constrained by complications involving low bioavailability, poor stability, and insufficient targeted distribution, underscoring the need for advanced delivery systems. Therefore, there is a strong need to develop an advanced topical drug delivery system that can improve the stability, enhance penetration, and provide sustained release of these phytoconstituents at the wound site. Incorporating these natural extracts into a novel formulation such as a nano-enabled topical gel can potentially overcome these barriers, maximize therapeutic efficacy, and offer a synergistic approach for improved wound healing outcomes.

## MATERIALS AND METHODS

### Materials

*Andrographis paniculata* powder was purchased from Annai Aravindh Herbals, Chennai, *Punica granatum* was procured from local garden, Lingasugur, Raichur District, Karnataka, Carbopol-940, Ethanol, Xanthan gum, Triethanolamine, Methyl paraben, Propyl paraben, Propylene glycol was purchased from Yarrow Chem Products, Mumbai. Sulphuric acid and Hydrochloric acid were procured from Rankem Chemicals. All other chemicals used were of AR grade.

### Collection and preparation of raw materials

The whole plant of *Punica granatum* was collected, powdered using mixer grinder, sieved through BSS# 85 and stored in closed containers for further extraction process.[10,11] *Andrographis paniculata* powder was collected and stored in closed containers for extraction process.

### Microwave-assisted extraction

10 g of *Punica granatum* powder was weighed and transferred into a beaker, 50 mL of 70% ethanol was added and the sample was exposed to microwave irradiation at conditions 50°C for 60 sec at 55 watts. The resulting sample was filtered, dried in a water bath and stored in air tight container. The same procedure was repeated for *Andrographis paniculata* powder.[12,13]

### Phytochemical analysis of the extract

Preliminary phytochemical screening of extract was carried out to identify the presence of major secondary metabolites such as flavonoids, alkaloids, tannins, steroids, and carbohydrates. Standard qualitative tests including Shinoda, alkaline reagent, Dragendroff's, Wagner's, Ferric chloride, Liebermann Buchard, Molisch and Fehling methods were employed based on characteristic color changes or precipitate formation, as described in previously reported studies.[14,15]

### GC/MS analysis *Punica granatum* and *Andrographis paniculata* extract

The ethanol extract was subjected to GC-MS analysis using a Clarus 680 GC equipped with a fused silica column (Elite-5MS). 1 µL sample was injected at an injector temperature of 220°C. The oven temperature program was: 50°C (2 min), ramped to 150°C at 15°C/min, held for 2 min, then ramped to 250°C at 30°C/min and held for 8 min. Mass detector conditions included an inlet line temperature of 250°C, ion source temperature of 230°C, and electron impact ionization at 70 eV.[16] Spectra were compared with the NIST (2014) library.

### *In silico* docking studies

*In silico* evaluation was performed using PyRx for docking and PyMOL for visualization, with protein structures obtained from RCSB Protein Data bank and ligands from PubChem.[17] The protein was prepared by removing water molecules, heteroatoms, and non-bonded hydrogens, and both protein (.pdb) and ligand (.sdf) files were converted to PDBQT format for docking. Binding affinity values with zero RMSD were considered, and the docked complexes were visualized to obtain 2D interaction images. The ligands identified for the study are as follows: 24-Norursa-3,12-diene, Dinaphtho[2,1-b:1',2'-d]furan, 5,6,8-Trimethoxydinaphtho[1,2-b:2',1'-d] furan, Anthraquinone, 1-(o-chlorophenyl), 1-Butanol, 3-methoxy-,Indoline, 2-(hydroxydiphenylmethyl)-,Tetrahydro-22-desoxy-tomatillidine,3-Hydroxy-6methoxyflavone, 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone,Anthraquinone,1-(o-chlorophenyl) and 3-Fluoro-5-trifluoromethylbenzoic acid,4-nitrophenyl ester.

## Estimation of tannins

Total tannin content was determined using Folin-Ciocalteu method. Phenolic compounds were extracted using suitable solvents (Methanol, Ethanol and Acetone) followed by filtration and diluted. And the extract was then reacted with Folin-Ciocalteu reagent (1: 10) and sodium carbonate, and incubated at room temperature for 30 to 60 min to form a blue complex. The absorbance of the developed color was measured at 765 nm using spectrophotometer. And the results were quantified using a calibration curve prepared with gallic acid.[18-20] The tannin concentration (in milligrams per Liter, mg/L) can be calculated using the formula:

$$\text{Tannin (mg/L)} = (\text{Absorbance - Intercept}) \times \text{Dilution Factor} / (\text{Slope of Standard Curve})$$

## Formulation of gel

The required quantity of xanthan gum/carbopol-940/HPMC K100 was dispersed in distilled water in a beaker and allowed to swell for 30 min. Propylene glycol, methyl paraben and propyl paraben was taken in another beaker and mixed with the extracts for 15 min. Nano silver was incorporated, followed by the addition of extract and preservative solutions into the gel base with continuous stirring for 15-20 min for homogenous gel.[21] The pH was then adjusted using triethanolamine. The formulation trials are shown in Table 1.

## Evaluation of gel formulations

### *Percentage yield*

Percentage yield was calculated by ratio of practical yield to theoretical yield. The following formula was employed in order to determine the percentage yield.[22-24]

$$\text{Percentage Yield (\%)} = (\text{Practical Yield} \times 100) / \text{Theoretical Yield}$$

## Appearance and homogeneity

Physical appearance and homogeneity of the gel formulation were assessed visually.

## pH measurement

pH was measured using a digital pH meter after dissolving 1 g of gel in 50 mL of water. The experiment was carried out in triplicate.

## Viscosity

Brookfield viscometer with spindle number 4 was used to evaluate the gel formulation's viscosity at 12, 30, and 60 RPM.[25]

## Spreadability

Spreadability was determined using a wooden block and pulley apparatus with 2 glass slides and standard weights. 1 g of gel was placed between two slides, compressed with a 1000 g weight for 5 min, after which the upper slide was allowed to move under a 20 g weight. The time taken to travel 7.5 cm was recorded, and spreadability was calculated using the standard formula:

$$S = m \times L/t$$

S - spreadability; m - mass applied in grams; L - length spread by the mass applied; t - time taken in seconds.

## Estimation of tannin content in gel formulation

100 mL of phosphate buffer were used to dissolve 1 g of gel formulation. After 15 min of bath sonication, the mixture was filtered. The tannin content was estimated by measuring the absorbance at 765 nm by UV-visible spectrophotometer (Shimadzu 1900) and calculated using a standard calibration curve.[26]

## *In vitro* drug permeation studies

The drug permeation was evaluated using Kishery-Chein diffusion cell. 500 mg of gel was placed between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer pH 7.4 and stirred at 100 rpm using a magnetic stirrer. At predetermined intervals, 1 mL samples were withdrawn and replaced with fresh buffer to maintain sink conditions. The samples were diluted, and absorbance was measured at 765 nm using a UV- visible spectrophotometer to estimate tannin content.[27]

## Acute dermal irritation study

The approval for animal studies was obtained from Institutional Animal Ethics Committee (IAEC) of Faculty of Pharmacy, M.S Ramaiah University of Applied Sciences. The reference number was XXIX/MSRFPH/CEU/PG-03/15.02.2024.

Acute dermal irritation study was carried out using New Zealand White rabbits of either sex, Following OCED guidelines 404. The dorsal surface was shaved and four regions of 6 $cm^2$ were marked, three were treated with the test gel formulation (F2) and one served as an untreated control. After 4 hr, residual gel was removed, and the animal was observed for erythema and oedema over 14 days. Based on dermal irritation scores, the formulation was classified as irritant (if effects persisted) or non-irritant (if no irritation was observed).[28]

## Excision model wound healing studies

The *Wistar rats* were maintained under laboratory conditions in standard cage for a week at the animal house. Cycle of 12 hr dark/light at 22±5°C and 60 to 70% relative humidity was maintained. The animals were fed with balanced diet and water supply was also provided. *In vivo* wound healing studies were carried out using *Wister rats* weighing about 200 to 250 g.[29-31] The rats were divided into 3 groups containing 3 animals each. Group 1 is control, group

2 is standard treatment with silver nitrate ointment and group 3 is formulation F2. The rats were anaesthetized via intraperitoneal route using ketamine (80 mg/kg) and xylazine (20 mg/kg). The dorsal region was depilated using a hair removal cream, and a 6 mm full- thickness excision was created in the interscapular area using a biopsy punch to evaluate wound contraction and epithelialization. The test formulation was applied daily until complete healing, and wound progression was monitored by measuring wound diameter on 3rd, 6th, 7th, 9th, 12th and 15th day.

### Histopathological studies

At the end of the treatment period, one rat from each group was anaesthetized, and the healed skin tissues were carefully excised and preserved in 10% v/v formalin. Sections of 3-4 cm in length were prepared by embedding them in paraffin and then haematoxylin and eosin was used for staining the embedded tissue for microscopic examination.[32] To analyse the biological parameters lipid profile and complete blood count.[33-34] were carried out.

### Stability studies

A 30-day accelerated stability study was carried out for F2 gel formulation in accordance with ICH recommendations, at the conditions 40±2ºC temperature and 75% RH.

## RESULTS

### Extraction Process

Microwave assisted extraction was performed to extract the phytoconstituents from *Punica granatum* and *Andrographis paniculata* crude drug. These methods were easy and have simple design, the process was carried out with low flow of solvent and maximum extraction yield was achieved in shorter time. The concentrated extract was weighed and percentage yield was calculated. The percentage yield was found to be 2.7% and 2.5% for *Punica granatum* and *Andrographis paniculata* respectively. Phytochemical analysis revealed the presence of alkaloids, carbohydrates, tannins, phenolic compounds and flavonoids as evidenced by Mayer's test, Dragendroff's test, Fehling's test, Ferric chloride test, Lead acetate test and Shinoda test.

### GC-MS analysis of the extract

GC-MS is used to separate complex extract mixtures by gas chromatography and to quantify and identify analytes by Mass chromatography. The obtained peaks of GC-MS analysis for *Punica granatum* and *Andrographis paniculata* are shown in Figures 1 and 2.

GC-MS analysis revealed the presence of five compounds in the sample. Indoline, 2-(hydroxydiphenylmethyl)- ($C_{21}H_{19}NO$) was identified at a Retention Time (RT) of 18.92 min and showed the highest peak area (14,599,406.0), indicating it as the major constituent. Tetrahydro-22-desoxy-tomatillidine ($C_{27}H_{47}NO$) was detected at 5.96 min with a peak area of 8,655,750.9 and the highest match factor (71.9). Anthraquinone, 1-(o-chlorophenyl)- ($C_{20}H_{11}ClO_2$) appeared at 7.42 min with a peak area of 1,314,464.5. 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone ($C_{17}H_{14}O_4$) was detected at 13.67 min with a peak area of 1,171,327.5, while 3-Hydroxy-6-methoxyflavone ($C_{16}H_{12}O_4$) was identified at 11.31 min with the lowest peak area (18,131.0). The identified compounds belong to different chemical classes, including alkaloids, flavonoids, chromenones, and anthraquinone derivatives, indicating the chemical diversity of the sample.

GC-MS analysis identified five major compounds in the sample. 5,6,8-Trimethoxydinaphtho[1,2-b:2',1'-d]furan ($C_{23}H_{18}O_4$) was detected at a Retention Time (RT) of 36.56 min and exhibited the highest peak area (17,416,902.0), indicating it as the predominant constituent. Anthraquinone, 1-(o-chlorophenyl)- ($C_{20}H_{11}ClO_2$) was identified at 7.16 min with a peak area of 10,001,725.7. 24-Norursa-3,12-diene ($C_{29}H_{46}$) appeared at 30.26 min with a

**Table 1: Formulation of Gel.**

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| *Andrographis paniculata* extract (g) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| *Punica granatum* Extract (g) | 1 | 1 | 1 | 1 | 1 | 1 |
| HPMC K 100 (g) | - | - | - | - | 2 | 3 |
| Xanthan gum (g) | 2 | 3 | - | - | - | - |
| Carbopol-940 (g) | - | - | 2 | 3 | - | - |
| Propylene glycol (mL) | 5 | 5 | 5 | 5 | 5 | 5 |
| Methyl paraben (g) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Nano Silver (mL) | 1 | 1 | 1 | 1 | 1 | 1 |
| Propyl paraben (g) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Triethanolamine | qs | qs | qs | qs | qs | qs |
| Rose Flavour | qs | qs | qs | qs | qs | qs |
| Distilled water (g) qs to | 50 | 50 | 50 | 50 | 50 | 50 |

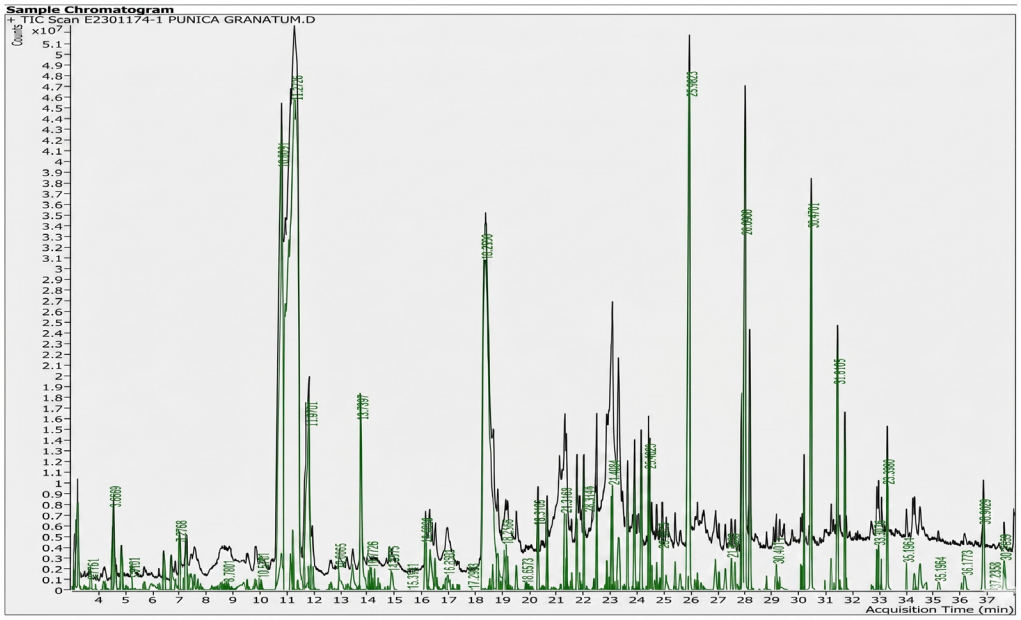



**Figure 1:** GC-MS chromatogram of *Punica granatum* leaf extract showing major phytoconstituents.

peak area of 2,447,785.9 and showed the highest match factor (74.6), indicating a relatively reliable identification. 1-Butanol, 3-methoxy- ($C_5H_{12}O_2$) was detected at 9.66 min with a peak area of 1,051,245.5, while Dinaphtho[2,1-b:1',2'-d]furan ($C_{20}H_{12}O$) was identified at 32.26 min with a peak area of 580,290.4. The identified compounds belong to diverse chemical classes, including triterpenoids, furan derivatives, anthraquinones, and alcohols, suggesting a chemically heterogeneous sample with potential biological significance.

## *In silico* docking studies

Phytochemical constituents from *Punica granatum* and *Andrographis paniculata* extracts were used as ligand molecules. Data on these constituents were obtained from literature. Compounds such as 24-Norursa-3,12-diene, Dinaphtho[2,1-b:1',2'-d]furan, 5,6,8-Trimethoxydinaphtho[1,2-b:2',1'-d]furan, and others showed superior results compared to the remaining tested compounds. Interactions of the compounds with proteins revealed conventional hydrogen bonding and Van der Waals forces. This *in silico* evaluation suggests that the phytochemical constituents of *Punica granatum* and *Andrographis paniculata* could be potent wound healing agents. For the protein 7aqg, the binding scores for *Punica granatum* extract was found to be Indoline, 2-(hydroxydiphenylmethyl) is -6.9; Tetrahydro-2 2-desoxy-tomatillidine is -6.8; 3-Hydroxy-6-methoxyflavone is -6.5; 5-Hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone is -6.4; Anthraquinone, 1-(o-chlorophenyl) is -6.3. The binding scores for *Andrographis paniculata* was found to be 24-Norursa-3,12-diene is -6.9; Dinaphtho[2,1-b:1',2'-d]furan is -6.7; 5,6,8-Trimethoxydinaphtho[1,2-b:2',1'-d]furan is -6.5; Anthraquinone, 1-(o-chlorophenyl) and 1-Butanol, 3-methoxy is -6.3.

## Estimation of Tannin content

Tannin was estimated for the obtained extract from microwave assisted extraction method. The tannin content was found to be 3.35 mg/2 g for *Punica granatum* and 3.9 mg/2 g for *Andrographis paniculata.*

## Evaluation Parameters for Gel

Percentage yield is used to assess the practical yield of the formulation and to identify any wastage or loss. It is computed as the percentage of the practical yield divided by the theoretical yield. This allows for a quantitative comparison of the expected versus actual output of the gel formulation. The results of physico-chemical evaluation of the gel are shown in Table 2.

## Appearance and Homogeneity

The color, homogeneity and phase separation of gel formulations were evaluated. Due to the brownish and green extracts used, the gels exhibited these colors. All formulations were observed to be uniform in appearance, with no phase separation, indicating a homogeneous mixture throughout.

## pH measurement

The average pH of all the prepared formulations was calculated and all were within the range of wound skin pH. The values of measured pH of the prepared gel formulation were found to in the range of 5.5 ±0.3 to 8.5 ±0.2. The results suggested that all formulations were found to have pH value closer to wound skin pH.

## Spreadability

The tendency of the gel to spread across the skin is referred to as spreadability. The uniform application of gel formulation on

the skin surface depends on its spreadability, which is inversely proportional to viscosity and influenced by rheological properties. Good spreadability ensures even distribution of the gel on the skin.

The spreadability of the formulated gels ranged from 0.6±0.4 to 1.5±0.6 g·cm/s. Among all formulations, F2 exhibited the highest spreadability (1.5±0.6 g·cm/s), indicating superior ease of application and better spreading characteristics. This was followed by F1 (1.33±0.5 g·cm/s), F6 (1.2±0.5 g·cm/s), F5 (1.1±0.5 g·cm/s), and F4 (0.8±0.5 g·cm/s), while F3 showed the lowest spreadability (0.6±0.4 g·cm/s). Overall, F2 demonstrated the most favorable spreadability profile among the tested formulations.

## Drug content of gel formulation

Tannin content in the gel formulation was estimated by employing UV-visible spectroscopy. The absorbance was checked at 765 nm for Tannins. The tannin content was higher in F2 in comparison to other formulations. The tannin content was found to be 2.7 mg, 3.0 mg, 2.5 mg, 2.3 mg, 2.4 mg and 2.0 mg for formulations F1-F6.

## *In vitro* drug permeation studies

The *in vitro* drug permeation studies data showed a cumulative drug release of 19.7% for F1, 20.8% for F2, 19.8% for F3, 18.7% for F4, 18.4% for F5, 18.2% for F6, respectively at the end of 6 hr.

## Acute Dermal Irritation Studies

The dermal irritation test was conducted to evaluate potential toxic effects following topical application of the formulation, in accordance with OECD Guideline 404. No signs of erythema, irritation or redness were observed throughout the study period. The observations of dermal irritation studies are shown Figure 3. This indicated that the F2 formulation is safe for topical application.

## *In vivo* wound healing studies

Wound healing was assessed by measuring wound area at predetermined intervals (days 3, 6, 9, 12, and 15) to evaluate wound contraction and epithelialization.[35] A significant increase in wound healing was observed in the F2 treated group compared to control and standard groups, with a notably higher rate of wound contraction, especially between days 6 and 15. Statistical analysis of wound contraction (p-value) is presented in Figures 4a and 4b.

Both marketed and test formulation groups show significant reductions in wound size compared to the control group from day 3 onwards. The test formulation shows a particularly high significance (***$p<0.0005$) in wound size reduction, especially from day 9 onwards. By day 15, the test formulation has almost completely healed the wounds (2±2.6), showing better efficacy than the marketed formulation.[36]

## Histopathology studies

The wound healing response in skin tissues was examined.[37,38] Disease control skin showed hyperkeratinisation with proliferation & follicular congestion hypodermal follicular hyperplasia with & inflammation which is moderate 3+ in marketed keratinocytes & ECM. Formulation F2 showed keratinocytes proliferated resulting in epithelialization & ECM restoration, active keratinocytes & ECM (Extracellular Matrix) proliferation induced healing response with vascularization of collagen layers which is extensive in entire dermis area. The images are shown in Figures 5a and 5b.

## Biological Tests

### *Lipid Profile Test*

Formulation F2 group showed higher total cholesterol (148 mg/dL) and triglycerides (85 mg/dL) compared to the control (135 and 70 mg/dL) and standard groups (141 and 65 mg/dL). HDL levels were slightly higher in the Test group (36 mg/dL) than Control (35 mg/dL) but lower than Standard (39 mg/dL). LDL was 60 mg/dL in the Test group, compared to 58 mg/dL (Control) and 65 mg/dL (Standard). VLDL was also elevated in the Test group (101 mmol/L) relative to Control (81 mmol/L) and Standard (93 mmol/L).[39]

The lipid profile analysis showed that the control group had total cholesterol, triglyceride, HDL, LDL, and VLDL levels of 135±2.1, 70±1.8, 35±1.2, 58±1.5, and 81±2.0 mg/dL, respectively. The standard (Silver Nitrate Gel) group exhibited values of 141±2.4, 65±2.0, 39±1.3, 65±1.7, and 93±2.3 mg/dL, respectively, with the highest High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels. The test formulation group showed the

**Table 2: Physicochemical evaluation of gel formulations.**

| Formulation | % Yield | pH (± SD) | Viscosity (cPs) @12 RPM | @30 RPM | @60 RPM |
|---|---|---|---|---|---|
| F1 | 90.4 | 6.6±0.2 | 177 | 123 | 112 |
| F2 | 91.2 | 6.8±0.1 | 210 | 160 | 103 |
| F3 | 89.3 | 5.5±0.3 | 176 | 125 | 104 |
| F4 | 84.5 | 5.8±0.2 | 78 | 42 | 29 |
| F5 | 76.6 | 8.5±0.2 | 45 | 17 | 16 |
| F6 | 72.5 | 8.2±0.2 | 43 | 21 | 15 |

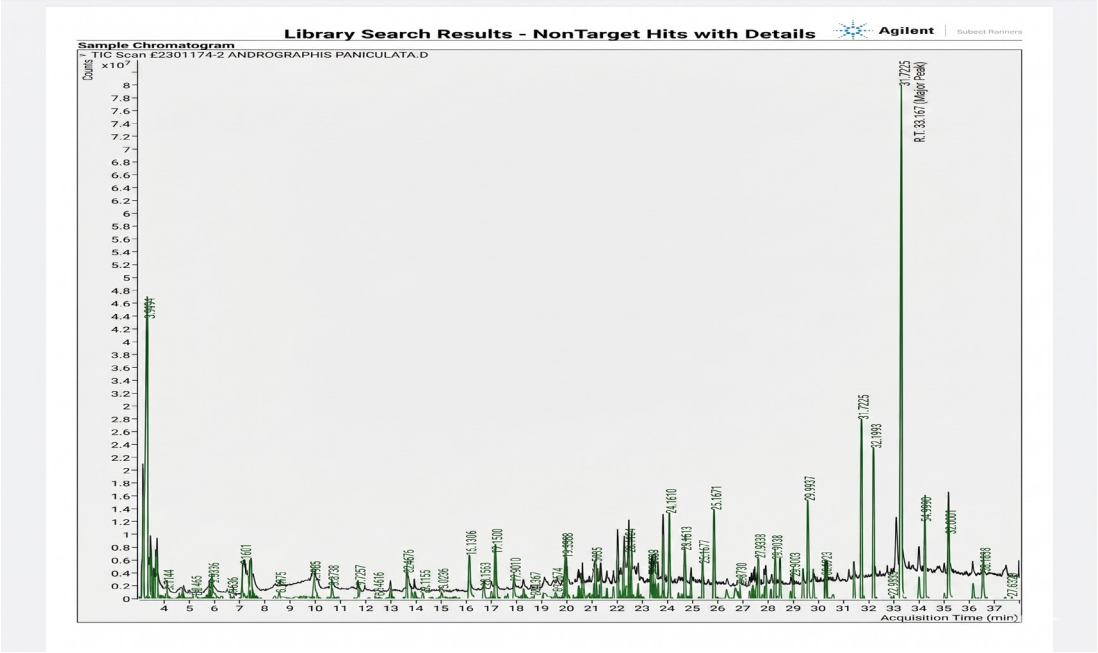



**Figure 2:** GC-MS chromatogram of *Andrographis paniculata* leaf extract showing major phytoconstituents.

highest total cholesterol (148±2.8 mg/dL), triglycerides (85±2.5 mg/dL), and Very Low-Density Lipoprotein (VLDL) (101±2.7 mg/dL) levels, while HDL and LDL levels were 36±1.4 mg/dL and 60±1.6 mg/dL, respectively. Overall, the test formulation resulted in higher cholesterol, triglyceride, and VLDL levels compared to the control and standard groups.

### Complete Blood Count (CBC)

Hemoglobin (10.5 g/dL) and hematocrit (31%) were slightly higher in the test group compared to control (10.3 g/dL, 30%) and standard (10.0 g/dL, 29%). Total cell count decreased in the test group (5,300 cells/µL) relative to control (10,300 cells/µL) and standard (6,300 cells/µL). RBC counts were similar in control and test groups that is 3.5 million/mm$^3$ in both, with a slight decrease in standard 3.4 million/mm$^3$. Platelet count was reduced in test group to 2.8 cells/µL compared to control with 3.8 cells/µL and standard 3.2 cells/µL. Neutrophils decreased (50%), while lymphocytes increased to 44% in test compared to control that is 60% and 34% and standard was 52% and 42%. Monocytes (4%) and eosinophils (2%) remained comparable between control and test groups. Overall, these variations indicate changes in hematological and immune status across groups (Tables 3 and4).[40]

### Stability Studies

The accelerated stability studies on the selected formulation F2 was carried out in accordance with ICH guidelines. The formulations demonstrated accepted stability, with no changes in physical appearance, pH, viscosity, or spreadability observed. The gel's physical appearance remained unchanged. After 30 days, the formulations were found to be homogeneous. The pH of the preparation was determined to be within the skin's pH range. The spreadability of the formulation did not differ significantly after the accelerated stability test. As a result, the developed gels were found to be stable.

## DISCUSSION

The present study focused on the development and evaluation of a topical gel containing extracts of *Andrographis paniculata* and *Punica granatum* incorporated with nano silver for enhanced wound healing activity. The selection of herbal components was based on their reported pharmacological properties, particularly antioxidant, anti-inflammatory, and antimicrobial activities, which are essential for effective wound management. Previous studies have demonstrated that phytoconstituents such as flavonoids and tannins play a significant role in promoting collagen synthesis, reducing oxidative stress, and accelerating tissue regeneration. Microwave-assisted extraction employed in this study proved to be an efficient technique for isolating bioactive constituents, yielding higher extractive values within a shorter duration. This method has been widely reported to enhance extraction efficiency by improving cell wall disruption and solvent penetration. Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, and alkaloids, which are known to facilitate wound contraction and epithelialization by modulating inflammatory responses and promoting fibroblast activity. Further, GC-MS analysis enabled the identification of various bioactive compounds, which may contribute to be observed therapeutic effects. The *in silico* docking studies provided additional support by demonstrating favourable binding interactions of selected compounds with proteins associated with proteins associated with wound healing pathways. Such findings are in accordance with recent reports

suggesting that molecular docking can effectively predict the biological activity phytoconstituents and their potential targets.

Among the six formulations developed, formulation F2 exhibited optimal physicochemical characteristics, including appropriate pH, viscosity, spreadability and maximum drug content. These parameters are critical in determining the performance of topical formulations. The pH of the gel was found to be within the acceptable range for skin application, thereby minimizing the risk of irritation. Adequate viscosity and spreadability ensure uniform application and prolonged residence time at the site of action, which are essential for improved therapeutic efficacy. The *in vitro* drug release studies indicated a sustained release pattern of the active constituents from the gel formulation. Sustained release is advantageous in wound healing applications as it maintains a consistent therapeutic concentration at the wound site, thereby reducing the frequency of application and enhancing patient compliance. Similar release profiles have been reported in nanoparticle based topical systems, where the presence of nanosilver facilities improved drug diffusion and retention. The *in vivo* wound healing studies using the excision wound model demonstrated a significantly enhanced rate of wound contraction in test group (F2) when compared to control and standard groups. The observed increase in wound contraction, particularly from day 9 onwards, may be attributed to enhanced collagen formation and rapid cellular proliferation. Faster epithelialization in the treated group further indicates effective regeneration of the epidermal layer. These findings are consistent with earlier studies reporting improved wound healing activity in formulations containing herbal extracts and metallic nanoparticles.

Histopathological evaluation revealed increased collagen deposition, neovascularization and well-organized epithelial layers in the treated group, confirming enhanced tissue repair and remodeling. Collagen plays a vital role in maintaining the structural integrity of the healed tissue, and its increased deposition indicates improved wound strength and stability. Biochemical parameters, including lipid profile, showed slight variations among the groups however, these changes were not significant and may be associated with physiological responses during the healing process. Haematological analysis indicated improved immune status and tissue repair activity in treated group, as evidenced by stable blood parameters. The dermal irritation study confirmed that the formulation was non-irritant and safe for tropical application. Furthermore, stability studies indicated that the optimized formulation remained stable under accelerated conditions, with no significant changes in its physicochemical properties, suggesting good formulation integrity and shelf-life.

Despite the promising findings, certain limitations of the present study should be considered. The experimental design involved a relatively small sample size, which may limit the generalizability of the results. In addition, the stability studies were conducted

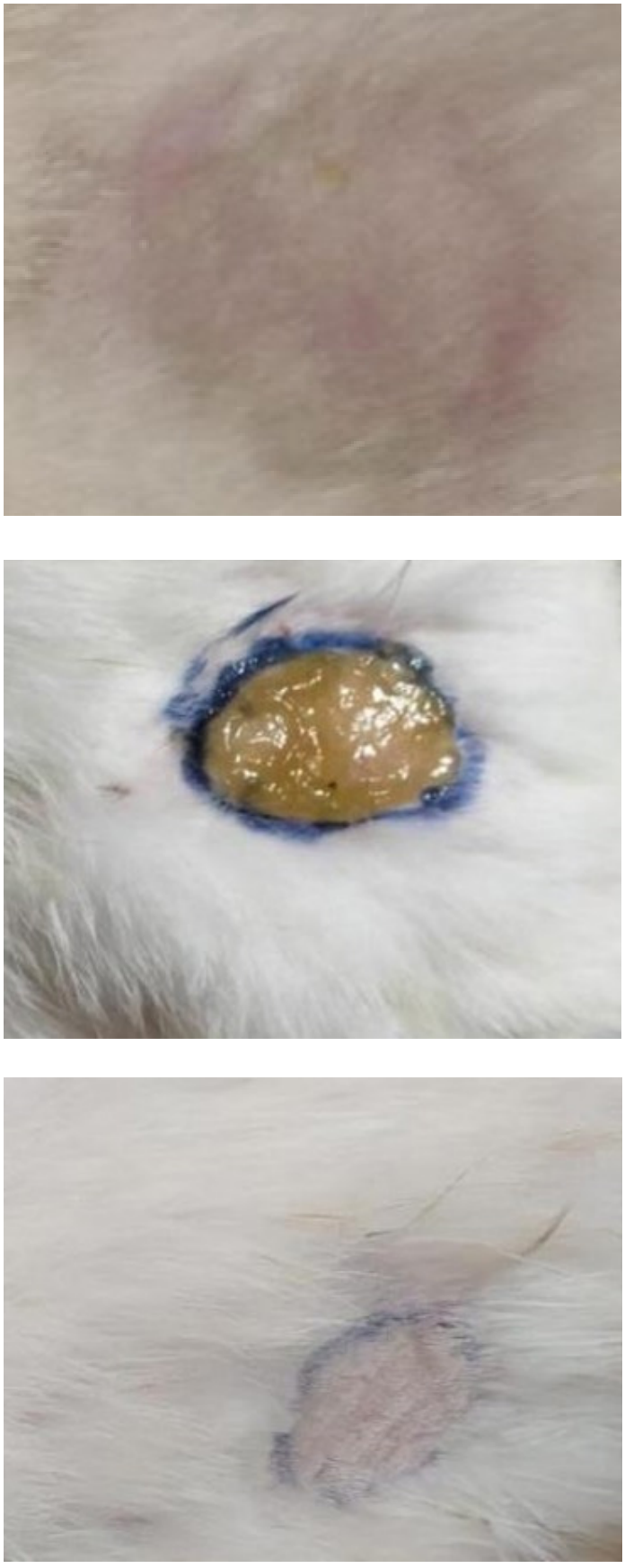


**Figure 3:** Skin irritation test of Formulation F2. (A) Before application, (B) After application, (C) After 4 hr of application.

**Table 3: Percentage wound area remaining.**

| Day | Control (%) mean±SD | Marketed (%) mean±SD | Formulation F2 (%) mean±SD |
|---|---|---|---|
| 0 | 100±0.0 | 100±0.0 | 100±0.0 |
| 3 | 85±1.8 | 80±1.4 | 78±1.1 |
| 6 | 70±2.5 | 57±2.0 | 54±1.5 |
| 9 | 55±3.2 | 37±2.5 | 29±2.0 |
| 12 | 40±3.9 | 17±3.0 | 10±2.4 |
| 15 | 30±4.2 | 5±3.3 | 2±2.6 |

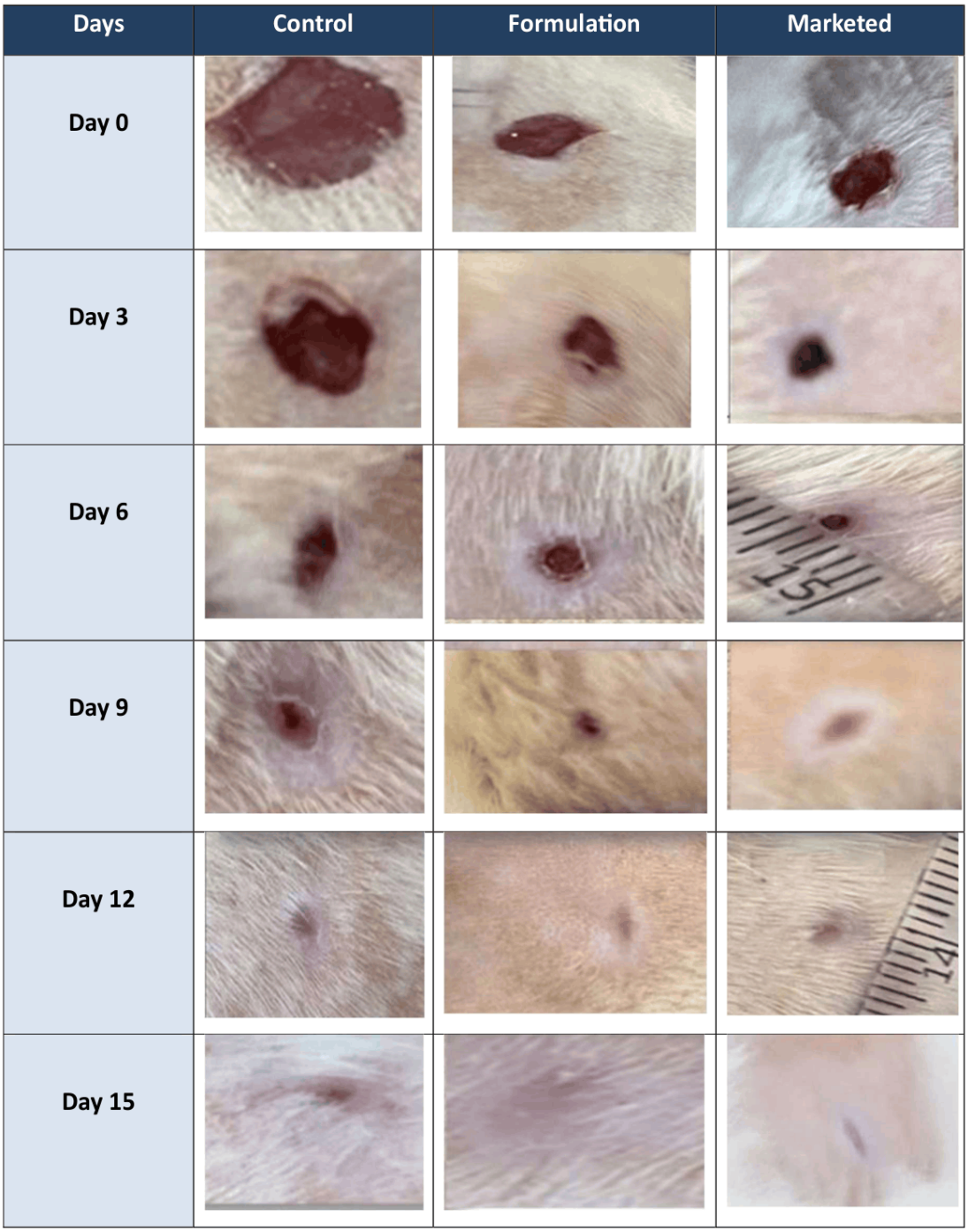
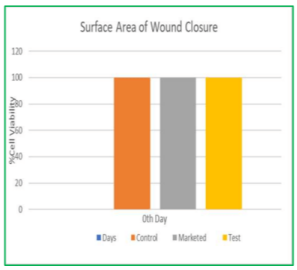
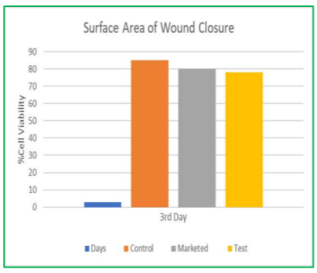


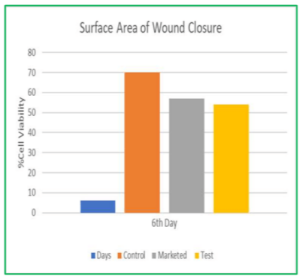

**Figure 4a:** Wound contraction of excision wound model.



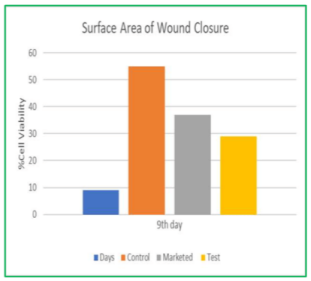

(a) Surface area of wound on 0th Day



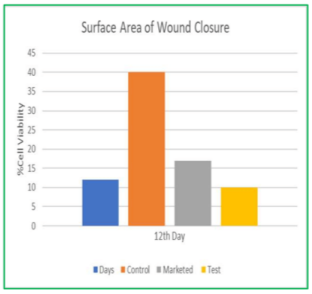

(b) Surface area of wound on 3rd day



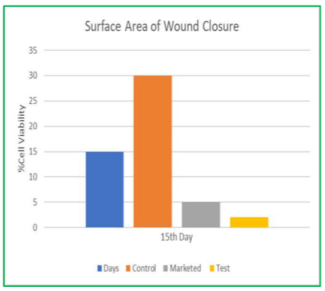

(c) Surface area of wound on 6th Day




(d) Surface area of wound on 9th Day




(e) Surface area of wound on 12th Day




(f) Surface area of wound on 15th Day

**Figure 4b:** Surface area of wound closure.

**Table 4: Report of Complete Blood Count (CBC).**

| Parameter | Control | Standard | Test Formulation |
|---|---|---|---|
| Hemoglobin (g/dL) | 10.3±0.4 | 10.0±0.3 | 10.5±0.5 |
| Hematocrit (%) | 30.0±1.2 | 29.0±1.0 | 31.0±1.3 |
| Total WBC Count (cells/µL) | 10300±420 | 6300±350 | 5300±300 |
| RBC Count (million/mm$^3$) | 3.5±0.2 | 3.4±0.1 | 3.5±0.2 |
| Platelet Count (×10$^5$ cells/mm$^3$) | 3.8±0.3 | 3.2±0.2 | 2.8±0.2 |
| Neutrophils (%) | 60±2 | 52±3 | 50±2 |
| Lymphocytes (%) | 34±2 | 42±2 | 44±3 |
| Monocytes (%) | 4±0.5 | 4±0.4 | 4±0.3 |
| Eosinophils (%) | 2±0.2 | 2±0.1 | 2±0.2 |

over a short duration, and therefore, long-term stability under varied storage conditions remains to be established. Furthermore, the molecular mechanisms underlying the observed wound healing activity were primarily inferred from *in silico* docking studies, which require further validation through detailed *in vitro* and *in vivo* investigations. Future studies should aim to address these limitations by conducting large-scale preclinical and clinical evaluations to substantiate the efficacy and safety of the formulation in diverse populations. Extended stability studies under different environmental conditions are necessary

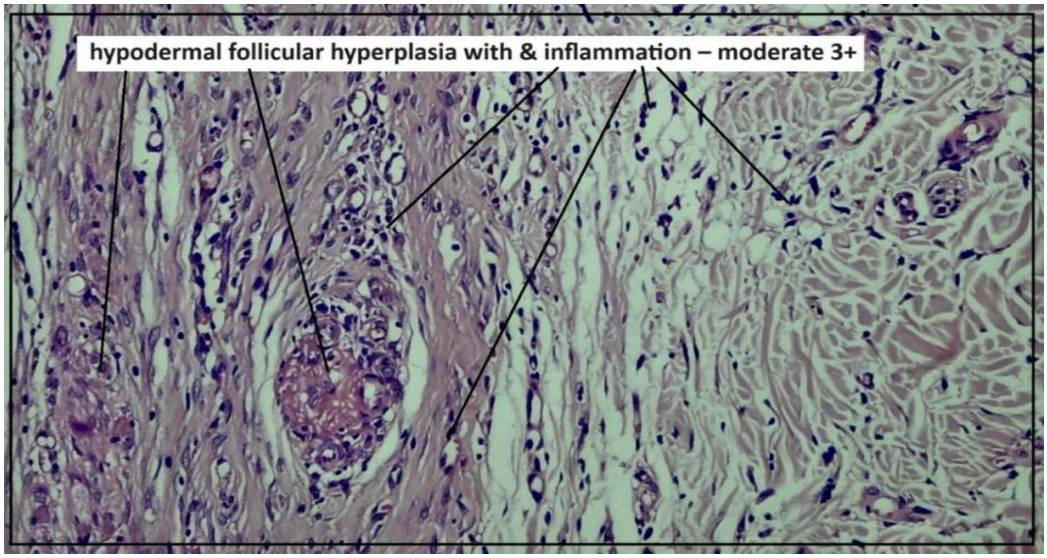



**Figure 5a:** Disease Control, Skin showing hypodermal follicular hyperplasia with & inflammation (100×).

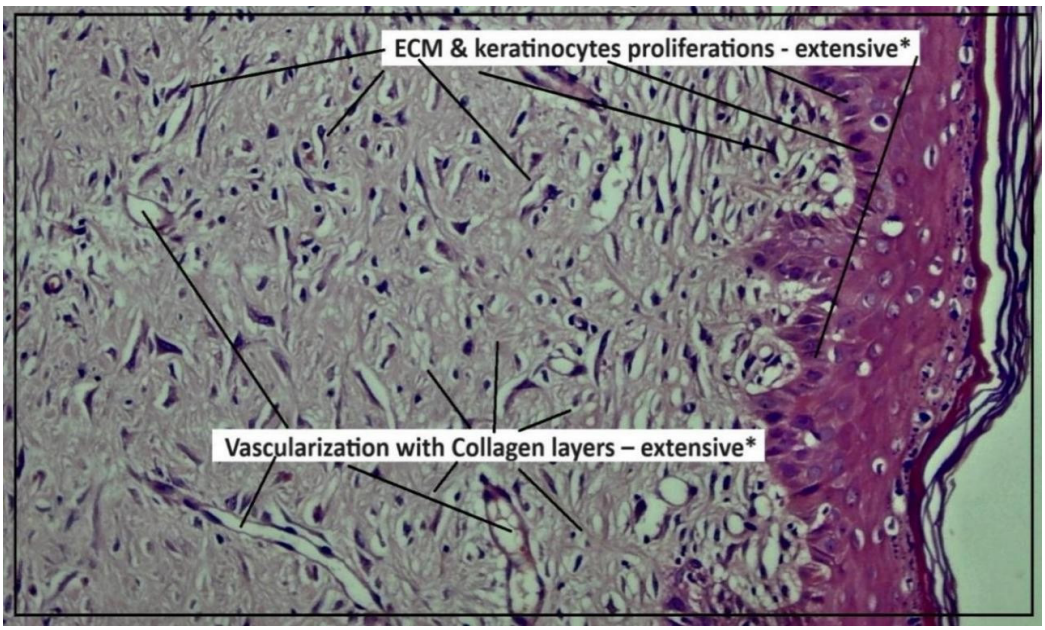



**Figure 5b:** Test Drug, Skin showing active keratinocytes & ECM proliferation's - healing response - Vascularization with Collagen layers (100×).

to confirm the robustness and shelf-life of the formulation. Moreover, comprehensive molecular and mechanistic studies should be undertaken to elucidate the exact pathways involved in the wound healing process. The integration of advanced drug delivery strategies, including targeted and stimuli-responsive systems, may further enhance the therapeutic performance and clinical applicability of the developed formulation.

## CONCLUSION

The study successfully developed a topical gel formulation containing *Andrographis paniculata* and *Punica granatum* extracts with nano silver, demonstrating significant wound healing activity. Among all formulations, F2 showed optimal physicochemical characteristics, effective drug release, and superior *in vivo* wound healing performance. The formulation promoted faster wound contraction, enhanced collagen deposition and improved tissue regeneration compared to control and standard treatments. Additionally, the formulation was found to be safe, non-irritant and stable under accelerated conditions. Overall, the findings support hypothesis that herbal based nano formulations can serve as effective and safe alternatives for wound healing applications. The developed gel holds promising potential for further clinical development and therapeutic use in wound management.

## ACKNOWLEDGEMENT

The authors are thankful to leadership team of RUAS and Dean, Faculty of Pharmacy, RUAS for providing the necessary support to carry out the work. The authors thank CITRA, Coimbatore for GC-MS analysis.

## ABBREVIATIONS

**GC-MS:** Gas Chromatography-Mass Spectrometry; **RMSD:** Root-Mean-Square Deviation; **PDBQT:** Protein Data Bank, Partial Charge (Q), and Atom Type (T); **HPMC:** Hydroxypropyl Methylcellulose; **UV:** Ultraviolet; **IAEC:** Institutional Animal Ethics Committee; **OECD:** Organisation for Economic Co-operation and Development; **ICH:** International Council for Harmonisation; **RH:** Relative Humidity; **ANOVA:** Analysis of Variance; **ECM:** Extracellular Matrix; **HDL:** High-Density Lipoprotein; **LDL:** Low-Density Lipoprotein; **VLDL:** Very Low-Density Lipoprotein; **WBC:** White Blood Cell; **RBC:** Red Blood Cell; **CBC:** Complete Blood Count.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

This research successfully integrated *Andrographis paniculata* and *Punica granatum* extracts with nano silver into a stable topical gel (Formulation F2). The formulation demonstrated optimal spreadability and sustained drug release, leading to significantly faster wound closure in animal models. Histopathological results confirmed enhanced tissue repair through improved collagen synthesis and vascularization.

## REFERENCES

1. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences. 2016;52(03):493-507.
2. Loureiro J, Miguel SP, Galván-Chacón V, Patrocinio D, Pagador JB, Sánchez-Margallo FM, Ribeiro MP, Coutinho P. Three-dimensionally printed hydrogel cardiac patch for infarct regeneration based on natural polysaccharides. Polymers. 2023;15(13):2824.
3. Guo SA, DiPietro LA. Factors affecting wound healing. Journal of dental research. 2010;89(3):219-29.
4. Bairy KL, Rao CM. Wound healing profile of Ginkgo biloba. J Nat Remedies. 2001;1:25-27
5. George Broughton II, Janis JE, Attinger CE. The basic science of wound healing. Plastic and reconstructive surgery. 2006; 117(7S):12S-34S.
6. Dai Y, Chen SR, Chai L, Zhao J, Wang Y, Wang Y. Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide. Crit Rev Food Sci Nutr. 2019;59:S17-29
7. Jurenka J. Therapeutic applications of pomegranate (*Punica granatum* L.): a review. Alternative medicine review. 2008;13(2).
8. Akbar S. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Altern Med Rev. 2011;16(1):66-77.
9. Dahanukar S, Kulkarni A, Rege N. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2000
10. El-Aguel A, Pennisi R, Smeriglio A, Kallel I, Tamburello MP, D'Arrigo M, *et al. Punica granatum* Peel and Leaf Extracts as Promising Strategies for HSV-1 Treatment. Viruses. 2022;14(12):2639
11. Chandrasekaran C V., Thiyagarajan P, Deepak HB, Agarwal A. *In vitro* modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of bitters) extract. Int Immunopharmacol. 2011;11(1):79-84.
12. Chia V V., Pang SF, Abdullah S, Yusoff MM, Gimbun J. Microwave-Assisted Extraction of Andrographolide, 14-Deoxy-11,12- didehydroandrographolide and Neoandrographolide from *Andrographis paniculata*: An Optimisation Study. Curr Nutr Food Sci. 2020;16(9):1318-25
13. Vasu S, Palaniyappan V, Badami S. A novel microwave-assisted extraction for the isolation of andrographolide from *Andrographis paniculata* and its *in vitro* antioxidant activity. Nat Prod Res. 2010;24(16):1560-7.
14. Keskin Cavdar H, Kocak Yanik D, Gok U, Goguş F. Optimisation of microwave-assisted extraction of pomegranate (*Punica granatum* L.) seed oil and evaluation of its physicochemical and bioactive properties. Food technology and biotechnology. 2017;55(1):86-94.
15. Maheshwaran L, Nadarajah L, Senadeera SPNN, Ranaweera CB, Chandana AK, Pathirana RN. Phytochemical Testing Methodologies and Principles for Preliminary Screening/Qualitative Testing. Asian Plant Research Journal. 2024;12(5):11-38.
16. Boadu A, Karpoormath R, Nlooto M. Spondias mombin: biosafety and GC-MS analysis of anti-viral compounds from crude leaf extracts. Advances in Traditional Medicine 2023;24(1):349-72.
17. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2015;1263:243-50.
18. Sayed Muhammad Amin Sadat, Anisa Omari. Extraction of tannins from *Punica granatum* peel for pharmacological activities. World Journal of Advanced Research and Reviews. 2020;8(1):144-7.
19. Mahboubi A, Asgarpanah J, Sadaghiyani PN, Faizi M. Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L. var. Pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases. BMC Complement Altern Med. 2015;15(1):366.
20. Ruiz-Vasquez L, Mesia LR, Maco MM, Zapata JA, Cotrina HR, Cobos M, *et al.* Optimized Folin-Ciocalteu Method for Determination of Total Polyphenols in Medicinal Plants of the Peruvian Amazon: Validation and Application to Twelve Species. AppliedChem. 2026;6(1):17.
21. Zode RD, Chakole CM. Formulation and Evaluation of Antibacterial Gel Using Leaf Extract of *Andrographis paniculata*. Indo American Journal of Pharmaceutical Research. 2020;10(6):2231-687.
22. Gannu R, Vamshi Vishnu Y, Kishan V, Madhusudan Rao Y. Development of nitrendipine transdermal patches: *In vitro* and *ex vivo* characterization. Curr Drug Deliv. 2007;4(1):69-76.
23. Munir M, Shah SNH, Almas U, Khan FA, Zaidi A, Bukhari SM, *et al.* An assessment of the wound healing potential of a herbal gel containing an *Azadirachta indica* leaf extract. Vet Med (Praha). 2021;66(3):99.
24. Upadhyay C, Pathak D, Kulshreshtha M. Preparation and evaluation of different herbal gels synthesized from Chinese medicinal plants as an antimicrobial agents. Pharmacological Research-Modern Chinese Medicine. 2023; 9:100313.
25. Lakhe E. *In vitro* assessment of anti-inflammatory activity of ethanolic extract of fenugreek seeds and demonstration of effectiveness in animal models (*in vivo*) through characterization of gel formulation containing the extract. *Int J Sci Res Publ*. 2021;11(6):571.
26. Martins GR, Monteiro AF, do Amaral FRL, da Silva AS. A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açaí (*Euterpe oleracea* Mart.) seeds extract. J Food Sci Technol. 2021;58(12):4693.
27. Mutalik S, Udupa N. Formulation development, *in vitro* and *in vivo* evaluation of membrane controlled transdermal systems of glibenclamide. J Pharm Pharm Sci. 2005;8(1):26-38.
28. ***28.*** OECD. Test No. 404: Acute Dermal Irritation/Corrosion. OECD Guidelines for the Testing of Chemicals, Section 4. 2015; OECD Guidelines for the Testing of Chemicals, Section 4.
29. Sun C, Yan H, Jiang K, Huang L. Protective effect of casticin on experimental skin wound healing of rats. Journal of Surgical Research. 2022; 274:145-52.
30. Ghanbari M, Salkovskiy Y, Carlson MA. The rat as an animal model in chronic wound research: An update. Life sciences. 2024; 351:122783.
31. De Moura Estevao LR, Cassini-Vieira P, Leite AG, de Carvalho Bulhoes AA, da Silva Barcelos L, Evencio-Neto J. Morphological evaluation of wound healing events in the excisional wound healing model in rats. *Bio Protoc*. 2019;9(13):e3285.
32. Slaoui M, Fiette L. Histopathology procedures: from tissue sampling to histopathological evaluation. In Drug safety evaluation: Methods and protocols 2010; 69-82. Totowa, NJ: Humana Press.
33. Pancholia AK, Kabra NK, Gupta R. Laboratory evaluation of lipid parameters in clinical practice. Indian Heart Journal. 2024; 76:S29-32.
34. Enos KE, Moore DM. Hematology of Laboratory Animals. Schalm's Veterinary Hematology, Seventh Edition. 2020; 1058-72.
35. Guideline IH. Stability testing of new drug substances and products. Q1A (R2), current step. 2003; 4(1-24).

36. Mukherjee P, Roy S, Ghosh D, Nandi SK. Role of animal models in biomedical research: a review. Laboratory animal research. 2022;38(1):18.
37. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Percoraro RE, Rodeheaver G, Robson MC. Definitions and guidelines for assessment of wounds and evaluation of healing. Wound repair and regeneration. 1994;2(3):165-70.
38. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. Journal of Investigative Dermatology. 2007;127(3):514-25.
39. Saghazadeh S, Rinoldi C, Schot M, Kashaf SS, Sharifi F, Jalilian E, Nuutila K, Giatsidis G, Mostafalu P, Derakhshandeh H, Yue K. Drug delivery systems and materials for wound healing applications. Advanced drug delivery reviews. 2018; 127:138-66.
40. Shabana MM, Gonaid MH, Salama MM, Sleem AA, Abdel-Sattar E. Lipid profile and biological activity of different extracts of *Stapelia hirsuta* L. Future Journal of Pharmaceutical Sciences. 2016;2(2):76-81.

**Cite this article:** Arpitha, Poojitha MG, Deveswaran R. Development of Topical Gel Containing Natural Extracts and Nano Silver for Wound Healing. Indian J of Pharmaceutical Education and Research. 2026; IPC Issue:15-27.