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Brahmidin: Canine defence against infections and inflammation

Ms. Aushima Dasari*, Ms. Nidhi Mishra, Mr. Nitesh Choudhary,Mr. Harish Birajdar, Mr. Pratham Jain, Mr. Dhiraj Gupta.

MET Institute of Pharmacy (Degree), Bandra Reclamation, Bandra (W), Mumbai, Maharashtra, India , 39554230.

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ABSTRACT

Wound infections and delayed healing are common challenges in veterinary care, particularly in canines. Conventional treatments, such as ointments and gels, often suffer from poor adhesion, frequent reapplication, and inconsistent drug delivery. To address these limitations, a spray adhesive patch was formulated using Curcuma longa (Turmeric), Azadirachta indica (Neem oil), and Centella asiatica, recognized for their antimicrobial, anti-inflammatory, and wound-healing properties in animal care.The formulation utilized bioethanol for improved drying control, ethyl cellulose for enhanced film formation, and a carefully balanced combination of glycerin and isopropyl myristate to prevent excessive stickiness and penetration. The spray was evaluated for spray uniformity, drying time, film adhesion, and stability, all of which met optimal standards for canine application. In-vitro diffusion studies confirmed controlled and sustained drug release, while the antimicrobial assay demonstrated a significant zone of inhibition against bacterial strains affecting canines. Additionally, an anti-inflammatory assay validated the formulation's ability to inhibit protein denaturation, supporting its efficacy in reducing inflammation and promoting wound healing.The optimized spray adhesive patch exhibited fast drying, strong adhesion, and enhanced therapeutic effects, making it a promising solution for efficient and effective canine wound management.

Keywords: Wound Healing, Canine, Spray Adhesive Patch, Antimicrobial, Controlled Drug Release

I. INTRODUCTION

1.1 Canine wound:

Wound healing in veterinary medicine is a complex, multi-phase process involving cellular and molecular interactions, often occurring simultaneously. Herbal remedies have long been used for treating animal wounds, with their effectiveness documented in ancient Indian Vedic texts. Key mediators like cytokines and chemokines play vital roles in regulating the healing process.

Stage 1: Haemostasis: Haemostasis involves immediate blood clotting via platelets and fibrin to stop bleeding. Emergency veterinary care is essential in the case of intense bleeding that is not going away(1).

Figure 1: Haemostatis

Stage 2: Inflammatory Phase: Inflammation is the body's natural response to injury, where white blood cells trigger swelling, warmth, and pain to aid healing. Deep or large wounds require veterinary care for cleaning, stitching, and proper treatment.[(1).]

Figure 2: Inflammatory phase

Stage 3: Proliferation Phase (repair phase): The healing phase involves wound contraction and granulation tissue formation through collagen, leading to closure. Owners should keep the area clean and watch for signs of infection like redness, swelling, or pus, which require veterinary attention.[(1).]

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Figure 3: Proliferation phase

Stage 4: Maturation Phase: Maturation is the final healing phase where collagen forms a scar that gradually flattens and shrinks. While no treatment is usually needed, the area may remain tender or reopen, possibly requiring veterinary care.[1].

Figure 4: Maturation phase

1.2. Spray Adhesive Patch for Canine Wound Healing

Traditional canine wound treatments like ointments and sprays often suffer from issues such as uneven application and reduced effectiveness due to licking, movement, or environmental factors. Spray adhesive patches offer a novel, non-invasive, and cost-effective alternative, ensuring uniform drug delivery, prolonged contact, and enhanced local bioavailability while minimizing systemic side effects. These patches form a protective layer that resists displacement and can easily incorporate potent herbal extracts, making them ideal for effective and sustained wound care in dogs[3][4].

Proteolytic enzymes and herbal extracts have been extensively used for their anti-inflammatory and wound-healing properties. In this formulation, a synergistic combination of **Curcuma longa, Azadirachta indica, and Centella asiatica** has been employed. **Curcuma longa** (turmeric) is known for its powerful anti-inflammatory and antimicrobial effects, helping to reduce infection and accelerate healing[5]. **Azadirachta indica** (neem) exhibits strong antibacterial, antifungal, and wound-healing properties, making it effective in preventing infections[6]. **Centella asiatica** promotes collagen synthesis, enhances skin regeneration, and strengthens the healing process[7].

1.3 Curcuma longa (Turmeric)

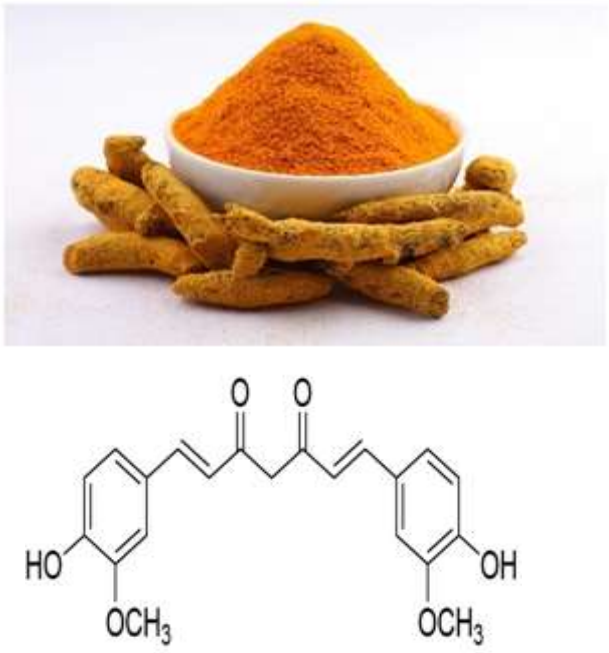

Figure 5: Curcuma longa and curcumin structure

Curcumin, a bioactive compound from turmeric, shows strong anti-inflammatory, antioxidant, and antimicrobial properties valuable in veterinary medicine. It aids in treating arthritis, dermatitis, and infections, supports wound healing, reduces fibrosis, and helps manage chronic conditions like diabetes and heart disease in pets. It also promotes liver health and cataract prevention, especially in older animals[8][9][10][11][12].

A Mechanistic Insight[13]:

Curcumin aids wound healing by suppressing pro-inflammatory cytokines (TNF-α, IL-1) and reducing oxidative stress via free radical scavenging and antioxidant enzyme activation. It enhances fibroblast activity, collagen synthesis, and granulation tissue formation during the proliferative phase. Curcumin supports angiogenesis and collagen remodeling, promoting faster wound closure with reduced scarring. Pharmacokinetic studies show low absorption and extensive hepatic metabolism, yet it maintains a good safety profile. Its bioactive properties make it suitable for veterinary wound management formulations.

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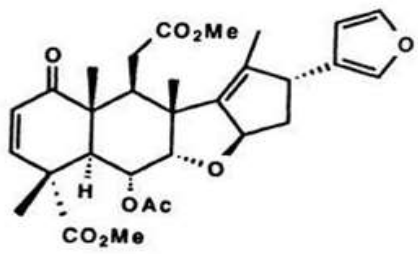
1.4 Azadirachta indica

Neem oil, derived from the seeds of Azadirachta indica, is a concentrated source of bioactive compounds such as azadirachtin, nimbin, and nimbidin, which exhibit potent antimicrobial, anti-inflammatory, and antifungal properties. Its high lipid content enhances dermal penetration and prolonged retention, facilitating deep wound healing and fibroblast proliferation essential for collagen synthesis and tissue regeneration. Unlike neem leaf extracts, neem oil provides a more effective barrier against pathogens while maintaining optimal moisture and antioxidant protection. In veterinary applications, neem oil has demonstrated accelerated healing, reduced inflammation, and improved tissue remodeling in canine wound models, positioning it as a superior phytotherapeutic agent for managing complex or chronic wounds.[14][15][16][17].

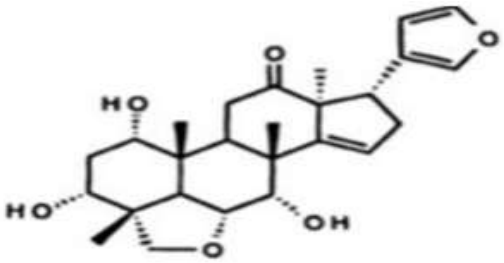
Figure 6: Neem oil

Neem's active compounds show moderate intestinal absorption (~55%) and a plasma unbound fraction of 0.318, indicating efficient membrane permeability. They exhibit low blood-brain barrier permeability (logBB < –1) and are primarily metabolized in the liver without notable CYP inhibition. The LD50 is high (~2000 mg/kg), reflecting a good safety profile.



nimbin

Figure 7: Nimbin



nimbidin

Figure 8: Nimbidin

1.5 Centella asiatica

Centella asiatica (L) Urban, known as Gotu kola, belongs to the family Apiaceae. This small perennial herb is widely distributed in Southeast Asia and forms part of traditional medicine that was practiced over 2000 years ago[18]. This herb is mentioned in ancient texts such as the "Sushruta Samhita". The plant has been a mainstay of traditional Chinese and Indian medicine for the treatment of various skin conditions like leprosy, varicose ulcers, and eczema[19]. Centella asiatica is reported to possess a rich phytochemical composition. Among others, it contains flavonoids, plant sterols, eugenol, and pentacyclic triterpenoids[20]. The most identified active component responsible for the drug's wound healing property is a pentacyclic triterpenoid saponin called asiatic acid, with the molecular formula $C_{30}H_{48}O_5$. This asiatic acid, being an aglycone of the saponin, has a complicated structure due to the presence of five rings in its molecular structure. The above structure empowers asiatic acid to interact with various biological targets, enhancing collagen synthesis and modulating inflammation, which are important in wound healing[21].

Figure 9: Centella asiatica

Asiatic acid has poor bioavailability because of its low water solubility, which is about 5.98 × 10-2 mg/L at 25°C; it is primarily absorbed

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from the jejunum. It binds with albumin and distributes through the tissues, including the plasma, brain, heart, liver, kidney, colon, and bladder. To increase its therapeutic value, chemical modifications have been tried to be carried out in an attempt to improve its aqueous solubility and bioactivity. Given the enormous therapeutic potential of C. asiatica and its derivative, asiatic acid, the application of these substances in veterinary medicine, particularly for cutaneous lesions in dogs, represents a particularly valuable resource[22]. Indeed, in this respect, asiatic acid has the peculiar property of enhancing collagen synthesis and modulating inflammation, thereby acting to speed the course of the healing process in dogs[23].

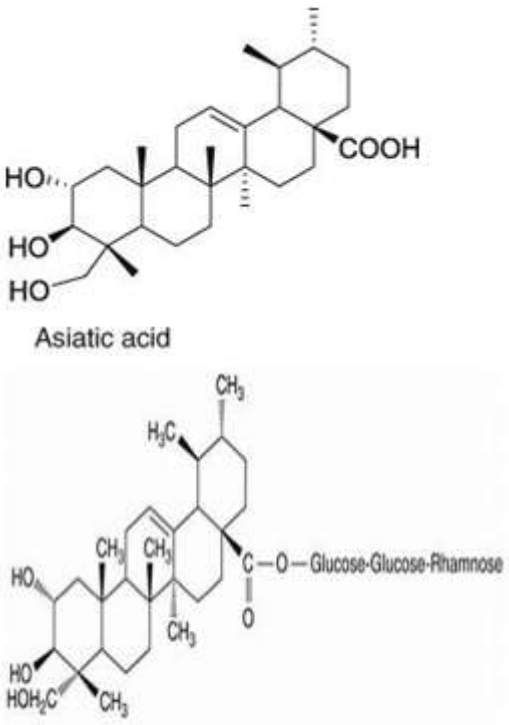

Figure 10: Asiatic acid & Asiaticoside

A Mechanistic insight

Centella asiatica extract (CAE) and its key triterpenoid, asiatic acid, exert multi-phase wound healing effects. In the **inflammatory phase**, they inhibit pro-inflammatory mediators (iNOS, COX-2, NF-κB, LOX) and reduce IL-17A/IL-23, limiting Th17-driven inflammation. During the **proliferative phase**, they stimulate fibroblast proliferation, angiogenesis, and synthesis of fibronectin and type I collagen. In the **remodelling phase**, CAE enhances ECM deposition via the TGF-β/Smad pathway, increases procollagen I/III expression, and promotes collagen maturation, improving tensile strength and wound closure[24][25].

The pharmacokinetic properties of Centella asiatica were studied using SwissADME and pkCSM. Centella asiatica shows moderate intestinal absorption due to its hydrophilic nature and high aqueous solubility. It has limited blood-brain barrier permeability with a plasma unbound fraction of 0.402. Metabolized mainly in the liver with minimal CYP inhibition, its high LD_{50} reflects a strong safety profile.

1.5. Objectives

1. The primary objective is to provide localized wound healing and pain relief for canines suffering from skin injuries and infections.
2. To promote the natural healing process and reduce inflammation by utilizing the synergistic effects of **Curcuma longa, Azadirachta indica, and Centella asiatica** in a spray adhesive patch formulation.
3. To create a protective barrier over the wound, shielding it from microbial contamination, environmental factors, and further injury.
4. To improve ease of application, ensuring better compliance and reduced stress for both the pet and the caregiver.

II. MATERIALS AND METHODS

2.1 Materials

Curcuma longa, Centella asiatica, and Azadirachta indica were used as actives. Neem oil (500 mL) and C. asiatica were procured from Yucca Enterprise (ICT Matunga), India, and C. longa from the local market. Excipients like ethyl cellulose, glycerine, isopropyl myristate, bioethanol, PVA, phosphate buffers, PEG-400, dichloromethane, chloroform, isopropyl alcohol, and glycerol were sourced from SD Fine Chemicals (SDFCL), Mumbai.

Egg albumin powder was obtained from Loba Chemie Pvt. Ltd., and fertilized hen eggs from Central Poultry Development Organisation (WR), Mumbai.

2.2. Methods

2.2.1. Preparation of curcumin extract

Curcuma longa (250 g) was powdered and extracted with 400 mL of ethanol using a Soxhlet apparatus for 24 hours. The extract was filtered, solvent recovered, evaporated, and dried in a desiccator. Final yield was weighed, and % yield calculated.;

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Percentage Yield: (Weight of Dried Extract / Initial Weight of Plant Material) ×100
Result: We successfully extracted Curcuma longa using the Soxhlet extraction method, obtaining a practical yield of 5% plant extract from the process.

2.2.2. Soxhlet extraction of Centella asiatica
Centella asiatica (250 g) was powdered and extracted with 400 mL ethanol using a Soxhlet apparatus for 24 hours. After filtration and solvent recovery, the extract was evaporated, dried in a desiccator, and weighed to calculate % yield.
Percentage Yield: (Weight of Dried Extract / Initial Weight of Plant Material) ×100
Result: We successfully extracted Curcuma longa using the Soxhlet extraction method, obtaining a practical yield of 2% plant extract from the process.

2.2.3. Preparation of reagents
2.2.3.a. Preparation of phosphate-buffered saline (PBS)
Dissolve 1.79 g of disodium hydrogen phosphate, 1.36 g of potassium dihydrogen phosphate, and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. pH is adjusted according to the requirements using a pH meter (ELICO LI 120 pH meter).

2.2.3.b. Preparation of phosphate buffer (PB), pH 6.8 (ref IP 2022)
Dissolve 28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml. The pH is adjusted according to the requirements.

2.2.3.c. Preparation of 1% albumin solution
1 g of egg albumin powder was dissolved in a minimal quantity of water. Lumps, if any, were broken down to obtain a homogenous solution. This solution was filtered through a cotton filter into a volumetric flask. Sufficient distilled water was added to produce 100 ml.

2.2.3.d. Preparation of 0.9% w/v sodium chloride
0.9 g of sodium chloride was weighed and transferred to a volumetric flask, and the volume was made up to 100 ml using distilled water.

2.2.4. Analytical evaluation
2.2.4.a. Detection of wavelength maxima (λmax)
Turmeric was first dissolved in distilled water to obtain a stock solution of concentration 100 mg/ml. This solution was scanned for λmax between the wavelengths 200 nm to 400 nm using UV/visible spectroscopy (Peak instruments, USA).

Centella asiatica was first dissolved in distilled water to obtain a stock solution of concentration 100 mg/ml. This solution was scanned for λmax between the wavelengths 200 nm to 400 nm using UV/visible spectroscopy (Peak instruments, USA).

Neem oil was first dissolved in ethanol to obtain a stock solution of concentration 100 mg/ml. This solution was scanned for λmax between the wavelengths 200 nm to 400 nm using UV/visible spectroscopy (Peak instruments, USA).

2.2.4.b. Calibration curve
After determining the λmax, solutions of varying concentrations were prepared and checked for linearity. Absorbance was determined at the λmax using UV/visible spectroscopy (Peak instruments, USA) and was plotted against their respective concentrations to obtain a linear graph.

2.2.5. Formulation Development and Optimization
Various formulations were made by observing the changes that were done, and the final formulation (F4) utilizes ethyl cellulose as the film-forming agent, with glycerine and isopropyl myristate for flexibility and penetration control. Bioethanol serves as the primary solvent, ensuring quick drying and minimal irritation. The active herbal extracts—Curcuma longa, Azadirachta indica, and Centella asiatica—were incorporated for their wound-healing and antimicrobial properties.

The polymers and excipients were dissolved in bioethanol and distilled water, with continuous stirring to achieve uniform solubility. The formulation was then homogenized using an ultrasonicator to ensure a stable dispersion of the ingredients. The final solution was transferred into a spray bottle, allowing for an even application onto the affected area. The spray was evaluated for adhesion, drying time, film formation, and therapeutic effectiveness to optimize its performance. However, the table below gives an overview of each formulation and its observation[26].

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Table 1: Overview of each formulation and its ingredients

Ingredients	F1	F2	F3	F4
Aloe vera extract	2.5%	-	-	-
Curcuma longa extract	2.0%	2.0%	2.0%	2.0%
Neem oil	2.5%	2.5%	2.5%	2.5%
Centella asiaticaextract	-	1.5%	1.5%	1.5%
HPMC	5%	-	-	-
Methanol	93%	-	-	
Ethyl cellulose	-	3.5%	3.8%	1.0% - 4.0%
Glycerine	-	2.5%	2.8%	3.0%
Isopropyl Myristate	-	2.0%	1.8%	2.0%
Isopropyl Alcohol (IPA)	-	86.0%	-	-
Bioethanol	-	-	85.6%	85.0%

2.2.6. Phytochemical evaluation

2.2.6.a Centella asiatica

Terpenoids were detected using the Salkowski test by observing a color change at the interface. Saponins were confirmed by stable foam formation. Dragendorff's test showed alkaloids via precipitate, while the Shinoda test indicated flavonoids through color change. TLC using a specific solvent system and anisaldehyde spray revealed compounds with calculated Rf values.

2.2.6.b Curcumin

Dragendorff's test was performed by adding Dragendorff's reagent to the extract to detect alkaloids. Lead acetate and ferric chloride tests were used to identify phenolic compounds, tannins, and flavonoids through precipitate formation or color change. Molisch's test involved layering sulphuric acid over the extract and Molisch's reagent to detect glycosides. TLC was performed too for further evaluation.

2.2.6.c Azadirachta indica

A TLC study was performed using a mobile phase of chloroform:acetone (3:1). The Azadirachta indica extract was spotted on a silica gel TLC plate, which was then placed in a pre-saturated chamber. After the solvent front reached three-fourths of the plate, it was removed, dried, and observed under UV light at 254 nm. The Rf value was calculated by measuring the distance travelled by the spot and solvent front.

2.2.7. Formulation Evaluation

These QC (Quality Control) tests are performed to ensure the consistency, effectiveness, and usability of spray formulations, such as pharmaceuticals, cosmetics, or industrial sprays. Here's why each test is conducted[27–29]:

The spray pattern and angle were assessed by spraying onto paper from 10–15 cm and measuring spread and angle using a ruler and protractor. Volume per spray and weight per mL were calculated by weighing sprayed amounts using a precision balance. Drying time was recorded until the surface was touch-dry. Washability was checked by rinsing the dried spray under water for 30 seconds. pH was determined using pH indicator paper.

2.2.8. In Vitro Diffusion Studies

In-vitro diffusion studies were performed using a Franz diffusion cell to evaluate drug release from the spray adhesive patch. A cellulose acetate membrane (soaked in PBS, pH 7.4) was used, with a diffusion area of 4.156 cm^2 and receptor volume of 22 ml. The study was conducted at 37 ± 1°C with constant stirring, and 2 ml samples were withdrawn every 15 minutes over 2 hours. Drug release of Curcuma longa, Azadirachta indica, and Centella asiatica was measured using calibration curves, and cumulative percent permeation was calculated and plotted against time[30].

2.2.9. Anti-microbial assay

The antimicrobial assay evaluated the spray's antibacterial activity using the disk

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diffusion method. Discs with the formulation were placed on bacteria-inoculated agar plates and incubated at 37 ± 1°C for 24 hours. Zones of Inhibition (ZOI) were measured in mm, and average values were recorded from triplicate tests(31).

2.2.10. Anti-inflammatory/Inhibition of protein denaturation assay

The Egg Albumin Denaturation Assay was performed to assess the anti-inflammatory activity of the spray by measuring its ability to inhibit protein denaturation. A reaction mixture of egg albumin, phosphate-buffered saline (pH 7.4), and the spray or standard (diclofenac) at various concentrations was prepared. After incubation at 37 ± 2°C for 30 minutes, samples were heated at 70 ± 2°C for 15 minutes to induce denaturation. Absorbance was measured, and percent inhibition was calculated to evaluate anti-inflammatory potential.[28].

$$\% \text{ inhibition} = \frac{AC - (AT - AB)}{AC} * 100$$

AC = Absorbance of control
AB = Absorbance of blank
AT = Absorbance of test

Table2: Ingredients to be added to the anti-inflammatory test

	Volume of 1% albumin (in ml)	**Volume of drug solution (in ml)**	**Volume of distilled water (in ml)**	**Volume of PBS pH 7.4 (in ml)**
Test	0.2	2	0	2.8
Blank	0	2	0.2	2.8
Control	0.2	2	2	2.8

2.2.11. Hen's Egg Test (HET) – Chorioallantoic Membrane (CAM) Assay

The HET-CAM test was conducted as an in vivo assay to evaluate the irritancy potential of the buccal patch. A 7–8-day-old fertilized hen's egg was prepared by exposing the chorioallantoic membrane (CAM) through the air cell after carefully removing the eggshell and inner membrane. The CAM was moistened and incubated at 37°C. A 1.5 cm patch was then placed on the CAM, and the onset times for haemorrhage, lysis, and coagulation were recorded to assess irritation. 0.9% sodium chloride served as the negative control and 0.1 N sodium hydroxide as the positive control. The Irritation Score (IS) was calculated based on these observations to classify the irritancy level of the formulation.[32].

Irritation score = (301- Ht)/300* 5+(301-Ct)/300*7 + (301-Lt)/300 *9
Ht- Haemolysis time
Ct- Coagulation time
Lt- Vessel Lysis time

Table 3: category and irritation score

Category	**Irritation Score**
Non-Irritative	0-0.9
Slightly Irritative	1-4.9
Moderately Irritative	5-8.9
Severely Irritative	9-21

2.2.12. Stability Testing

The stability studies evaluated the physical, chemical, and microbiological integrity of the film-forming spray to confirm shelf life, storage conditions, and formulation effectiveness. Two approaches were used: an accelerated study (at 40°C ± 2°C and 75% RH ± 5% RH for 1 month) and a real-time study (at ~25°C for 3 months). Key parameters assessed included appearance, viscosity, spray uniformity, drying time, and pH to ensure consistent performance and stability throughout the product's intended use.[33].

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III. RESULTS

3.1 Analytical Evaluation – Determination of λmax and Calibration Curve

The stock solutions of Curcumin, Centella asiatica, and Neem oil were scanned using a UV/Visible spectrophotometer. The maximum absorbance (λmax) values were found to be 420 nm, 413 nm, and 282 nm, respectively. Calibration curves were constructed using subsequent dilutions.

- For Curcuma longa, linearity was established within the concentration range of 0.1 – 0.5 mg/mL, with a slope of 0.1811, an intercept of 0.0143, and $R^2 = 0.9862$, indicating a strong linear correlation.
- For Centella asiatica, linearity was observed within the same range, with a slope of 0.1353, an intercept of 0.1119, and $R^2 = 0.9932$, indicating excellent correlation.
- For Neem oil, the linear regression line yielded a slope of 0.0566, an intercept of 0.118, and $R^2 = 0.9985$, demonstrating strong linearity.

The concentration vs. absorbance data for the phytoconstituents confirmed the linear relationships used for quantitative analysis.

3.2 Selection of Spray

The spray formulations were evaluated and optimized in successive stages:

- **F1** was rejected due to instability and safety concerns linked to methanol. Film formation was inadequate.
- In **F2**, methanol was replaced with **IPA**, and **ethyl cellulose** and **glycerin** were added to improve film-forming and moisturizing properties.
- **F3** involved replacing IPA with **bioethanol** and optimizing excipients for reduced stickiness and better penetration.
- **F4** demonstrated **optimal adhesion, flexibility, drying time**, and **therapeutic efficacy** and was selected as the final optimized formulation.

3.3 Phytochemical Evaluation

3.3.a Centella asiatica

- **Salkowski Test**: A reddish-brown color at the interface indicated the presence of **triterpenoid glycosides**.
- **Foam Test**: A stable foam (~1 cm) confirmed the presence of **saponins**.
- **Dragendorff's Test**: No precipitate formed; **alkaloids absent**.
- **Shinoda Test**: Color change observed; **flavonoids absent**.
- **TLC**: A brown-violet spot at **Rf = 0.3** indicated the presence of **Asiaticoside**.

3.3.b Curcuma longa

- **Dragendorff's Test**: Red coloration confirmed the presence of **alkaloids**.
- **Lead Acetate Test**: A red precipitate indicated **phenolics, flavonoids, and tannins**.
- **Molisch Test**: A violet-red ring indicated **glycosides**.
- **Ferric chloride Test**: Color change confirmed **phenolic compounds**.
- **TLC**: Yellow fluorescent spots under UV light revealed **curcuminoids**, with a major spot at **Rf = 0.6**.

3.3.c Azadirachta indica

- **TLC**: A visible spot at **Rf = 0.6** under 254 nm UV light confirmed the presence of active phytochemicals.

3.4 Organoleptic Evaluation

The optimized spray (F4) showed the following physical characteristics:

Table 4: Organoleptic evalution's result

Parameter	Observation	Remarks
Appearance	Clear, smooth film	Uniform, no particles
Colour	Light yellow to pale green	Due to turmeric and neem
Odour	Mild herbal	Non-irritating
Consistency	Free-flowing liquid	Easy sprayability
Spray Feel	Non-sticky, quick-drying	Comfortable and safe
Application Effect	Forma thin film <1 min	Protective barrier
Ease of Use	Easy, even distribution	User-friendly

Additional tests:

- **Spray Pattern**: Uniform and consistent.
- **Spray Angle**: 80°, symmetrical distribution.
- **Volume per Spray**: 0.148 mL.
- **Weight per mL**: 0.918 g/mL.
- **Drying Time**: 1–2 minutes.

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- **Washability**: Non-washable; strong adhesion.
- **pH**: 6.0 (slightly acidic).

3.5 In Vitro Diffusion Study

Cumulative drug release after 6 hours showed:

- **Neem oil**: 86.86%
- **Curcuma longa**: 41.38%
- **Centella asiatica**: 35.88%

Neem oil exhibited the fastest release due to **lipophilicity and low molecular weight**, suitable for rapid **antimicrobial action**. Curcumin showed a **moderate release**, beneficial for sustained **anti-inflammatory action**, while Asiaticoside released **slowly**, supporting **collagen synthesis and healing**.

3.6 Antimicrobial Assay

The **1:1:1** ratio of extracts exhibited the most effective antimicrobial activity:

Table 5: Result of Antimicrobial Assay

Sample	E. coli	Bacillus	Streptococcus	Klebsiella
Curcuma longa	6.2 ± 0.3	7.0 ± 0.4	6.5 ± 0.2	5.8 ± 0.3
Centella asiatica	5.9 ± 0.2	6.4 ± 0.3	6.0 ± 0.2	5.5 ± 0.2
Neem oil	7.0 ± 0.4	7.5 ± 0.3	6.8 ± 0.3	6.2 ± 0.3
Combined (1:1:1)	**14.8 ± 0.5**	**15.6 ± 0.4**	**14.5 ± 0.5**	**13.9 ± 0.4**
Other ratios	ND	ND	ND	ND
Control (Chloramphenicol)	15.6 ± 0.4	18.4 ± 0.3	16.8 ± 0.5	14.2 ± 0.6

Only the **1:1:1** combination produced clear zones of inhibition.

3.7 Anti-Inflammatory Assay

The protein denaturation inhibition test showed dose-dependent activity:

Table 6: Result of Anti-Inflammatory Assay

Conc. (µg/mL)	Avg. Absorbance	% Inhibition
100	0.286	15%
200	0.358	31%
300	0.464	**95%**
400	0.432	51%
500	0.564	79%

Maximum inhibition was observed at **300 µg/mL**, indicating strong anti-inflammatory potential.

3.8 HET-CAM Assay

The irritation potential was evaluated, and the **Irritation Score (IS)** was calculated.

Table 7: Result of CAM Assay

Test Substance	H (sec)	L (sec)	C (sec)	IS	Category
Negative Control (Saline)	>300	>300	>300	0.0697	Non-Irritant
Positive Control (NaOH)	9.6	78.3	300	10.08	Severe Irritant
Formulation	**>300**	**>300**	**>300**	**0.0699**	**Non-Irritant**

The formulation was classified as **non-irritant**.

3.9 Stability Study

The film-forming spray-maintained stability under both **accelerated and real-time conditions**. No significant changes were observed in **appearance, viscosity, spray uniformity, pH, or drying time**, confirming **physical, chemical, and microbiological stability**. Room temperature storage and protection from sunlight were recommended. Extended studies beyond **12 months** were suggested for further shelf-life enhancement.

IV. DISCUSSION

The project successfully led to the formulation of a spray adhesive patch (F4) containing Curcuma longa, Centella asiatica, and Azadirachta indica for canine wound healing. F4 exhibited optimized film adhesion, flexibility, and

drying time, along with enhanced wound healing and antimicrobial efficacy, while minimizing irritation, stickiness, and excessive penetration.

The formulation demonstrated excellent organoleptic properties, including a uniform spray pattern, an 80° spray angle, pH 6 (skin-compatible), and a quick drying time of 1–2 minutes. It passed all physical evaluations, with a spray volume of 0.148 mL and a weight per mL of 0.918 g/mL. It was non-washable, ensuring prolonged contact with the wound site.

In vitro diffusion studies showed the highest drug release for Neem oil (86.86%), followed by Curcuma longa (41.38%) and Centella asiatica (35.88%), enabling both rapid and sustained therapeutic action. The formulation exhibited significant anti-inflammatory activity (95% inhibition) and strong antimicrobial action in a 1:1:1 ratio, with zones of inhibition comparable to standard antibiotics.

The HET-CAM assay confirmed it as non-irritant (IS: 0.069). Overall, F4 proved to be a stable, safe, and effective topical formulation for canine wound care, combining herbal actives for synergistic anti-inflammatory, antimicrobial, and healing effects.

V. CONCLUSION

In conclusion, the formulation of the spray adhesive patch containing Curcuma longa, Centella asiatica, and Neem oil demonstrated promising results for wound healing in canines. The optimized formulation, F4, exhibited excellent film adhesion, flexibility, rapid drying, and balanced excipients, ensuring adequate wound coverage without irritation. The formulation showed significant antimicrobial activity, controlled drug release, and enhanced wound healing potential. The non-irritant nature and superior film-forming properties suggest that the developed spray adhesive patch could be a practical and effective alternative to conventional wound care treatments for canine skin injuries.

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Conflict of Interest

The authors declare no conflict of interest related to this work.

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