Formulation and Evaluation of *Cocculus Hirsutus* Mucilage Gel For Anti Inflammatory and Wound Healing Activities.

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ABSTRACT

Natural polymers derived from medicinal plants have gained increasing attention in topical drug delivery because of their biocompatibility, safety, and therapeutic potential, natural polymers made from medicinal plants have drawn more and more attention in topical drug administration. The creation and assessment of a mucilage-based topical gel made from *Cocculus hirsutus* for its anti-inflammatory and wound-healing properties was the goal of this study. Plant mucilage was isolated and used as the main natural gelling agent; to increase gel stability and consistency, a small amount of Carbopol was added. To ascertain whether the produced formulation known as Coccuheal gel—was suitable for topical administration, it underwent a number of physicochemical tests, including pH, viscosity, spreadability, extrudability, and homogeneity.

Compatibility studies were performed using Fourier Transform Infrared Spectroscopy (FTIR) was used to conduct compatibility experiments between the plant extract, Carbopol , and the produced Coccuheal gel. The results verified that there were no notable chemical interactions between the formulation's constituent parts. Trypsin inhibition and in vitro protein denaturation techniques were used to assess the gel's anti-inflammatory properties. Significant anti- inflammatory potential was demonstrated by the formulation's 36.76% inhibition in the protein denaturation method and 65.09% inhibition in the trypsin inhibition assay. Additionally, the formulation's wound healing activity showed 9.25% activity in the in vitro wound healing model, indicating its capacity to promote tissue repair and regeneration.

The mucilage-based gel that was created demonstrated both potential biological activity and acceptable physicochemical properties. According to the study's findings, Coccuheal gel, which is primarily made of natural mucilage and contains very little synthetic polymer, may be used as a herbal topical formulation to treat inflammatory diseases and promote wound healing. However, additional clinical research and in vivo studies are needed to verify its safety and therapeutic effectiveness.

Keywords: Topical herbal formulation, wound healing, anti-inflammatory properties, mucilage gel and *Cocculus hirsutus.*

INTRODUCTION

Inflammation is a complicated biological reaction of bodily tissues to damaging stimuli like irritants, viruses, or damaged cells[1]. Prolonged or unchecked inflammation can result in a number of pathological problems, such as chronic wounds, skin illnesses, and tissue damage, even though it is a preventive mechanism. Non-steroidal anti- inflammatory drugs (NSAIDs) and other conventional anti-inflammatory drugs are frequently used to treat inflammatory conditions, but long-term use of these medications is frequently linked to side effects like gastrointestinal irritation, renal problems, and delayed wound healing[2]. The creation of safer and more potent therapeutic co-mpounds made from medicinal plants is therefore gaining popularity[3].

Herbal medicines have been widely used in traditional medical systems have made extensive use of herbal remedies because of their availability, safety, and therapeutic efficacy. Bioactive substances originating from

plants,including flavonoids, alkaloids, phenolics, and saponins have been shown to have important anti-inflammatory, antioxidant, and wound healing qualities[4]. By encouraging collagen production ,angiogenesis , andre-epithelialization during the wound healing process, these phytoconstituents aid in tissue regeneration[5]

Cocculus hirsutus (family: Menispermaceae) the perennial climbing plant *Cocculus hirsutus* is found throughout tropical and subtropical countries and has long been used in Ayurvedic and traditional medicine to treat fever , inflammation , skin conditions and other illnesses[6]. According to phytochemical research, the plant's pharmacological actions are caused by a number of bioactive substances, including as alkaloids, flavonoids, steroids, and phenolic components. *Cocculus hirsutus* has the potential to be a therapeutic medicinal plant because recent pharmacologicalstudies have shown that extract contain antioxidant , immunomodulatory , antiviral , and anti-inflammatory qualities.[7]

Experimental studies have also shown that additionally, research has demonstrated that *Cocculus hirsutus* extract can inhibit signaling pathways such NF-κB and Src/Syk kinases to limit inflammatory mediators and lower nitric oxide production, indicating its potential use in inflammatory illnesses[8]. Additionally, the plant has shown biological activities that are crucial for wound healing and tissue repair, such as collagenase inhibition and antioxidant properties[9].

Topical gel formulations are widely preferred for the treatment of skin inflammation andwounds due to its simplicity of administration, enhanced drug penetration, patient compliance, and targeted therapeutic action, topical gel formulations are frequently chosen for the treatment of wounds and skin irritation[10]. Because they are more affordable, non-toxic, and biodegradable than synthetic polymers , natural polymers derived from plant mucilage have garnered interest as potential gelling agents[11]]. Therefore, adding extracts from medicinal plants to a mucilage-based gel system can improve therapeutic efficacy while reducing the need for artificial excipients [12] . The current study sought to develop and assess a topical gel of *Cocculus hirsutus* based on mucilage in light of these factors[13]. In order to investigate the produced formulation's potential as a natural topical medicinal agent, its physicochemical characteristics were described and its anti-inflammatory and wound healing properties assessed[14]

MATERIALS AND METHODS

Plant material, Reagents and Equipments

The study utilizes ***Cocculus hirsutus***, a natural plant material sourced from the Coimbatore herb garden,specifically for its recognized **anti-inflammatory and wound healing** properties. To transform this botanical material into a stable pharmaceutical gel, a variety of reagents are employed: **Carbopol** (Reachem) serves as the primary gelling agent.The liquid phase of the gel includes **Glycerine** (Reachem) and **Propylene glycol** (Molychem) acting as a humectants and **Ethanol** (Precision Scientific) as the primary solvent. To ensure the formulation's safety and longevity, **Methyl paraben** (Nice Chemical) is added as a preservative, and **Triethanolamine** (Spectrum) is utilized as a pH adjustor. The preparation and evaluation process are carried out using specialized equipment, including an **Ohaus Analytical balance** for precise weighing, **Borosil beaker** for mixing, and both **Remi** magnetic and mechanical stirrer to achieve homogeneity of the herbal gel.

Preparation of Plant Extract

The leaves of *Cocculus hirsutus* were harvested from Coimbatore, Tamil Nadu. Following collection, the specimens underwent a rigorous cleaning process with distilled water to remove exogenous contaminants and soil particles. The leaves were then subjected to controlled shade-drying at ambient temperature to prevent the thermal degradation of volatile oils and thermolabile phytoconstituents. Once dehydrated, the material was pulverized into a uniform fine powder using a mechanical grinder and stored in moisture - resistant, airtight containers to maintain its chemical stability. The extraction was performed using a cold maceration technique to ensure maximum cellular exhaustion of the plant matrix. Approximately 3 g of the powdered drug was submerged in 90 mL of ethanol, establishing a drug-to-solvent ratio of 1:30 (w/v). This mixture was maintained in a sealed glass vessel at room temperature for a duration of 48 hours. During this period, the

vessel was agitated intermittently to facilitate the diffusion of secondary metabolites into the menstrum. Following the 48-hour incubation, the mixture was strained through a muslin cloth, followed by secondary filtration using Whatman No.1 filter paper to obtain a clear filtrate. The resulting solution was concentrated using a water bath at a regulated temperature below 50°C until a consistent semi-solid crude extract was achieved. A designated portion of this extract was preserved for preliminary phytochemical screening to detect various plant constituents, while the remaining yield was utilized as the active botanical ingredient for the formulation of experimental gel batches.

Phytochemical screening for plant extract

S.No	Phytochemical Test	Test Name/ Reagents	Procedure	Observation	Inference
1.	Flavonoids	Lead Acetate Test	Add 2–3 drops of 10% lead acetate solution to1–2mL of plant extract and shake gently.	Formation of a white precipitate	Presence of Flavonoids.
2.	Saponins	Foam Test	Add 2mL of distilled water to 1ml of plant extract and shake vigorously for 30 seconds.	Formation of persistent foam layer.	Presence of Saponins
3	Coumarin	Alkaline (NaOH) Test	Add 1 ml of 10%NaOH solution to the Plant extract.	Formation of a yellow colour.	Presence of Coumarin.
4	Steroids	SalkowskiTest	Mix 2 ml extract with 2 ml chloroform; carefully add 2 ml concentrated sulfuric acid along the side	Formation of a reddish-brown colour.	Presence of Steroids.
5	Glycosides	Wagner'sTest	Add a few drops of Wagner's reagent and dilute iodine solution to 2 ml of plant extract.	Formation of reddish brown precipitate	Presence of Glycosides.

Table 1: Procedure for Phytochemical Screening

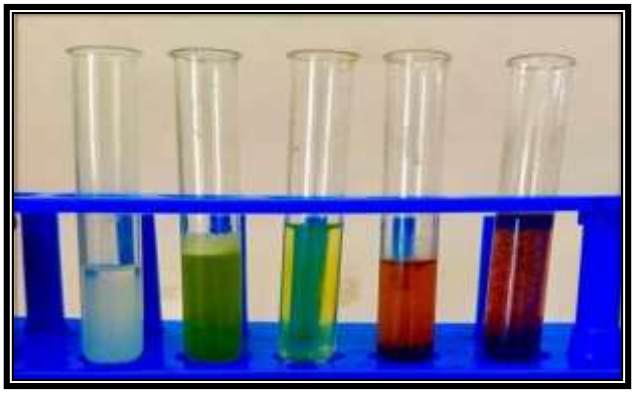

Figure 1: Phytochemical Screening test

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Formulation Procedure

For the preparation of CoccuHeal gel (F1–F4), the required amount of *Cocculus hirsutus* powder was accurately weighed for each batch. Distilled water was added, and the mixture was stirred thoroughly before being allowed to swell at room temperature for 1–2h, forming a homogeneous mucilage gel that served as the natural gelling agent. Separately, Carbopol 940 was weighed and hydrated with distilled water overnight. The Cocculus mucilage gel and the hydrated Carbopol gel were then combined and stirred slowly to obtain a uniform polymer base. To this base, glycerine and propyleneglycol were added along with methyl paraben as a preservative,and the mixture was stirred thoroughly to ensure uniform distribution. The *Cocculus hirsutus* extract was dissolved in ethanol and incorporated slowly into the polymer gel while stirring gently to achieve a homogeneous gel. Finally, triethanolamine was added dropwise to neutralize the gel, and stirring was continued until complete gel formation and a final pH of 5.5–6.5 was achieved.

GELCODE	**F1**	**F2**	**F3**	**F4**
Cocculus hirsutus extract	2g	2g	2g	2g
Cocculushirsutusmucilage(hydrate)	1.5g	2.5g	3.5g	4.5g
Carbopol940(hydrate)	2.5g	4.5g	6.5g	8.5g
Glycerine	3ml	3ml	3ml	3ml
Propyleneglycol	1ml	2ml	3ml	4ml
Ethanol	2ml	2ml	2ml	2ml
Methylparaben	0.02g	0.02g	0.02g	0.02g
Triethanolamine	0.05g	0.05g	0.05g	0.05g

Table 2: Formulation Procedure

F1 **F2** **F3** **F4**

Figure 2: Formulation of gel

METHODOLOGY FOR EVALUATION OF GEL

Characterisation of *cocculus hirsutus*

The characterization is done to identify the characteristics functional groups of *cocculus hirsutus* (l.) extract, carbopol 940 and final coccuheal gel to evaluate compatibility between extract, polymer and excipients.

FTIR - Fourier Transform Infrared Spectroscopy Procedure

Equipment: FTIR Spectrophotometer (JASCO, Bruker)

Scanrange: Typically recorded in the mid-infrared range,often 4000–400cm^{-1}

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Procedure

Place the KBr pellet into the sample holder.Run a backgrounds can (air or pureKBr) to eliminate ambient interference.Scan the sample to generate the spectrum.

Evaluation Parameters

1. Physical appearance: In this test, the gel was observed for color, odour , texture and state.

2. P^H: Measurement of the gel was carried out using a digital pH meter (Systronics) p^H SYSTEM 361 by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out intriplicate and the average of the three readings was recorded.

3. Viscosity: The viscosity of the gel was determined using a Brookfield viscometer (S-62, model LVDV-E) at 25°C with a spindle speed of the viscometer rotated at 60 rpm.

4. Spreadability: To conduct a Spreadability test using a texture analyzer, start by firmly attaching the suitable probe, like a cone probe, to the machine. Next, prepare the sample by placing it evenly inside the cone-shaped. Carefullly lower the instrument's arm, ensuring the probe is directly above the sample.Centre the sample and adjust the probe to be a few millimeters above the surface. Once everything is correctly positioned, initiate the test, allowing the probe to penetrate and spread the sample. The instrument will record the force needed for spreading, yielding important information about the texture and consistency of the sample.

5. Extrudability: A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end, and a clamp was applied to prevent any rollback. The cap was removed, and the gel was extruded. The amount of the extruded gel was collected and weighed, and the percentage of the extruded gel was calculated.

6. *In-vitro* anti inflammatory: In –vitro anti-inflammatory studies test substances outside a living organism-using cell cultures or biochemical assays -to measure their ability to inhibit protein denaturation, stabilize membranes, reduce pro-inflammatory enzymes (COX, LOX), lower cytokines and the Methods todetermine if a compound has anti-inflammatory properties are

Diclofenac sodium method- A Protein denatuation Assay

To evaluate the anti-inflammatory activity of Diclofenac,a protein denaturation assay was performed. Initially, two test samples were prepared using 1.0 ml and 0.5 ml of Diclofenac, each combined with 3 ml of Phosphate Buffered Saline (PBS) and 1 ml of a1% egg albumin solution.These mixtures were incubated at37°c for 20 minutes to allow for initial interaction.Following incubation, the samples were heated at 90^0C for 2 to 3 minutes to induce protein denaturation.After cooling to room temperature, the optical density(OD) was measured at 660 nm to quantify the extent of inhibition compared to a control.

Formula used for the analysis

$$\text{INHIBITION\%} = \frac{AC - AT}{AC} \times 100$$

Trypsin method: To begin the assay, 1 ml of the sample was taken and 0.06mg of trypsin was added along with 1ml of 20 mM Tris-HCI buffer.The solution was mixed thoroughly and incubated at 37°c for 5 minutes. Following this, 1 ml of 0.8% casein solution was added, and the mixture was incubated again for 20 minutes. To stop the reaction, 2 ml of 70% perchloric acid was added.The resulting mixture was centrifuged at 5000 rpm for 5 minutes. Finally, the supernatant was collected and the optical density(OD) was measured at a wavelength of 210nm, with a secondary reference reading taken at 280 nm.

Formula used for the analysis

$$\text{INHIBITION\%} = \frac{C - T}{C} \times 100$$

***In –vitro* Wound Healing :** Grow cells in DMEM with high glucose media supplemented with 10% FBS until the cells reach 70-80% confluence. Seed cells into 12 well tissue culture plate at a density of 0.25 million cells per well, until they reach—80- 100% confluence as a monolayer for the incubation period of 24hrs.Do not change the medium. Gently and slowly scratch the monolayer with a new 200ul pipette tip across the center of the well. While scratching across the surface of the well, the long-axial of the tip should always be perpendicular to the bottom of the well. The resulting gap distance therefore equals to the outer diameter of the end of tip. The gap distance can be adjusted by using different types of tips. Scratch a straight line in one direction Scratch another straight line perpendicular to the first line to create across in each well. After scratching, gently wash the well twice with medium to remove the detached cells. Treat the cells with desired concentrations of given compound prepared in fresh basal media and incubate at 37°c with 5% C02 in the incubator. Grow cells for additional 24-48hr (or the time required if different cells areused). Capture the cell images at different time intervals (ex:0, 24hr, 48hrs etc.,) Set the same configurations of the microscope when taking pictures for different views of the monolayer. The gap distance can be quantitatively evaluated using software such as Image. To reduce variability in results, it's suggested that multiple views of each well should be documented, and each experimental group should be repeated multiple times.

Formula used for the analysis:

% of Wound Healing Scored = (Initial area – Final area) / Initial area * 100

RESULTS AND DISCUSSION

Characterization of *Cocculus hirsutus*

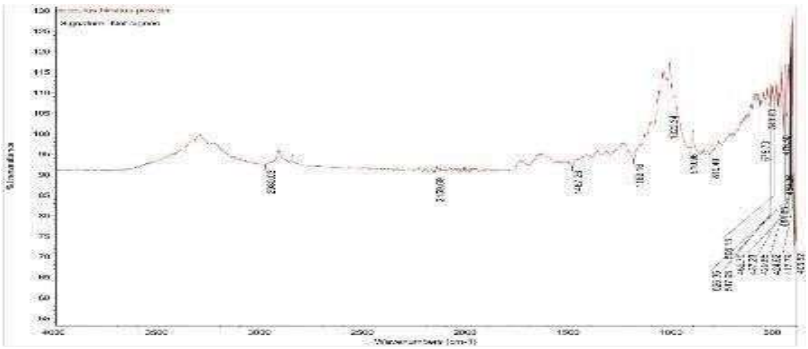

Figure 3: FTIR graph for *Cocculus hirsutus* extract

Wave number	FunctionalGroup	Intensity
3300	O−Hstretching	Broad
2980.03	O−Hstretching	Weak
2159.69	C≡CorC≡N	Sharp/Weak
1487.26	C=Cstretching	Medium
1189.16	C=Cstretching	Medium

1022.34	C−Ostretching	Strong/ Sharp
910.96- 819.49	C–O−Cstretching	Weak
526.35- 405.52	Fingerprintregion	MultipleSharp

Table 3: FTIR Analysis of Cocculus hirsutus extract

The Characteristic Peaks Stretching 0-H[Phenols, Alcohol], C-H[Aliphatic Ch2Or Ch3 Groups], C≡C Or C≡C [Alkyne or Nitrile Groups], C=C [Aromatic Ring], C-O-C [Polysaccharides],C-H Bending [Aromatic] which is evident that these peaks are confirming the *Cocculus Hirsutus* extract.

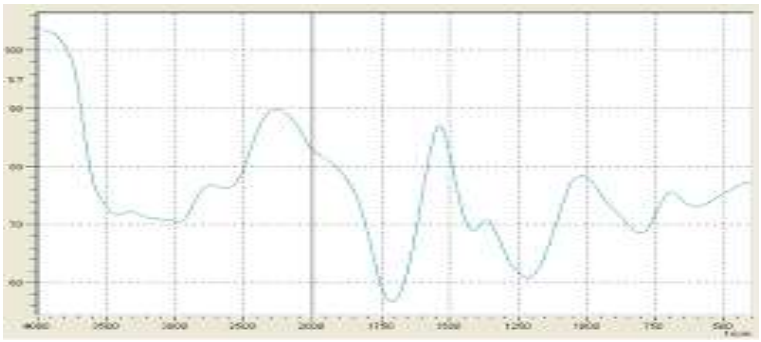

Figure 4: FTIR graph for Carbopol

Wavenumber	Functional Group	Intensity
3400–3000	-OH (HYDROXYL)	Stretching
2930	C-H (ALIPHATIC)	Stretching
1710	C = O (CARBONYL)	Stretching
1450	C–O/O-H	Bending
1245	C−O	Stretching
1160	C-C	Stretching

Table 4 : FTIR Analysis of Carbopol

The Characteristic Peaks Stretching-OH [Hydrogen bonding and carboxylicacid] ,C-H [CH2 groups in the poly acrylic acid], C=O [carboxylic acid group COOH], C-O / O-H [Scissoring and bending vibrations], C-O[C-O stretching of the acid and cross-linking ether groups], C-C[Skeletal vibrations of the acrylate] which is the evident that these peaks are Carbopol 940.

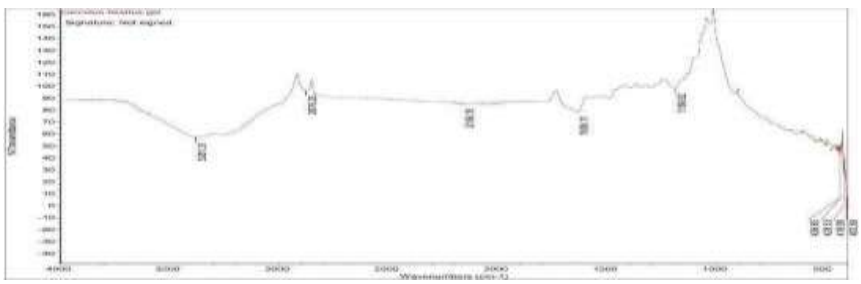

Figure 5: FTIR graph for CoccuHeal gel

Wavenumber	Functional Group	Intensity
3381.31(drug)	O–HStretching	Strong,Broad
2876.25(drug)	C–HStretching	Medium
2158.15(drug)	C≡C/N=C=S	Weak
1636.11(polymer)	C =OorC=N	Medium/Sharp
1188.62(polymer)	C−Ostretching	Weak/Broad
436.65-403.59	FingerprintRegion	SharpClusters

Table 5: FTIR Analysis for CoccuHeal gel

The FTIR Studies conclude that there is no major interaction between extract polymer and excipient.

Evaluation of gel

Physical Evaluation

In this test color, odor, texture and state of the four formulation were checked.

PARAMETERS	F1	F2	F3	F4	F4
Colour	Faint green	Faint green	Faint green	Faint green	Faint green
Homogenity	Good	Good	Good	Good	Good
Texture	Smooth	Smooth	Smooth	Hard	Hard
State	Liquid	Semi solid	Semi solid	Solid	Solid

Table 6: Physical evaluation of gel

2. p^H: The p^H of formulation F3 was found to be close to the skin's natural pH, indicating that it can be safely used on the skin.The p^H of formulation F3 is 6.57.

3. **Viscosity**

Rpm	%torque	Centi Poise(cps)
0.6	79.3	158600
1.0	79.3	95200
1.5	79.3	95200

Table 7: Viscosity Analysis of gel

According to the above results for formulation F3 showed an adequate viscosity.

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4.Spreadability

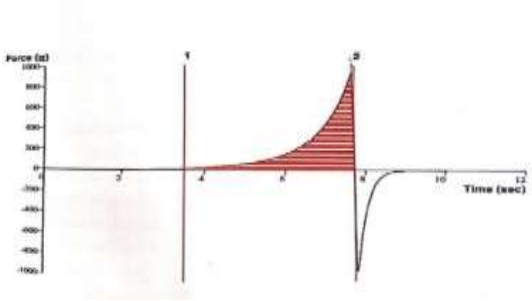


Figure 5: Spreadability Test

Spreadability for the formulation F3 was carried out and showed better spreadability.

4. Extrudability:

Extrudability for the formulation F3 was carried out and showed better gel extrudability

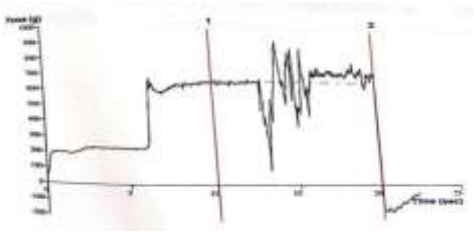


Figure 6: Extrudability Test

5. In-vitro anti–inflammatory:

1. Diclofenac sodium method / Protein denaturation method :

$$\text{INHIBITION\%} = \frac{AC - AT}{AC} \times 100$$

$$= \frac{0.680 - 0.430}{0.680} \times 100$$

$$= 36.76\ \%$$

2. Trypsin method:

$$= 65.09\ \%$$

6. Woundhealing :

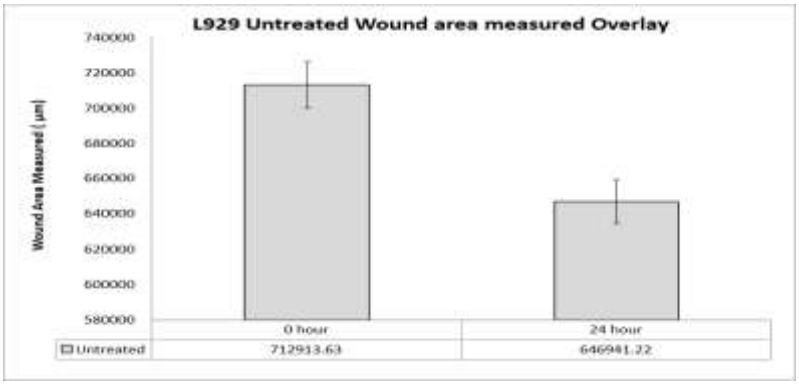
SUMMARY			
L929–UNTREATED WOUND AREA MEASURED OVERLAY			
INCUBATION	**AREA**	**INITIALAREA–FINALAREA**	**%WOUND HEALING SCORED**
0Hour	712913.63	0.00	0.00
24Hour	646941.22	65972.41	9.25

Table 8: Wound healing summary

FORMULA

% of Wound Healing Scored = (Initial area – Final area) / Initial area * 100

=712913.63 – 646941.22 / 712913.63× **100**



=9.25%

Table 9 : Wound healing measured

Sample – 0 hr

Sample – 24 hrs

Std – 0 hr

Std – 24 hr

Control -0 hr

Control -24hrs

CONCLUSION

The present study successfully formulated and evaluated a mucilage based topical gel of *Cocculus hirsutus* for anti- inflammatory and wound healing activities. The ethanolic extract was prepared and characterized, and FTIR studies confirmed compatibility between the extract, Carbopol and other formulation components, indicating no significant drug- excipient interaction.The formulated gel F3 showed satisfactory physic chemical properties, including acceptable pH, appropriate viscosity, good homogeneity, spreadability and extrudability, confirming its suitability for topical application. The in-vitro anti-inflammatory study demonstrated significant inhibition, showing 36.76% activity by protein denaturation (diclofenacsodium)

method and 65.09% inhibition by trypsin method. The formulation also exhibited 9.25% wound healing activity, indicating its potential to promote tissue repair. Overall,the results suggest that the *Cocculus hirsutus* mucilage gel is a promising and stable herbal formulation with potential therapeutic benefits in the management of inflammatory and wound conditions. Further in-vitro and clinical studies are recommended to substantiate its efficacy and safety.

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Conflict of Interest

We Declare That We Have No Conflict of Interest.

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