World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

PHARMACOLOGICAL EVALUATION, ANTI-OXIDANT AND WOUND HEALING ACTIVITY OF POLYHERBAL FORMULATION CONTAINING LEAF EXTRACTS OF *EUPHORBIA HIRTA* AND *BRYOPHYLLUM PINNATUM*

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DOI: https://doi.org/10.5281/zenodo.17748689

How to cite this Article: Abdul Kalam*, Rahul Dubey, Dr. Bala Subramanian**.** (2025). Pharmacological Evaluation, Anti-Oxidant and Wound Healing Activity Of Polyherbal Formulation Containing Leaf Extracts of Euphorbia Hirta And Bryophyllum Pinnatum. World Journal of Pharmaceutical and Life Science, 11(12), 164–172.

Article Received on 24/10/2025 **Article Revised on 14/11/2025** **Article Published on 01/12/2025**

ABSTRACT

The present study aimed to develop and evaluate topical herbal gels containing *Euphorbia hirta* and *Bryophyllum pinnatum* extracts for their phytochemical profile, antibacterial activity, and wound healing potential. Qualitative phytochemical screening confirmed the presence of active constituents such as alkaloids, flavonoids, glycosides, saponins, phenols, and proteins in both extracts. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was carried out using gallic acid and rutin as standards, respectively. Acute toxicity studies revealed no signs of toxicity up to 2000 mg/kg body weight, indicating the safety of the extracts. The prepared gels exhibited acceptable physicochemical characteristics, with pH values ranging from 5.9 to 6.6, suitable viscosity, and good spreadability without causing skin irritation. Antibacterial evaluation by well diffusion assay demonstrated that the polyherbal gel (Formulation III) exhibited the highest zone of inhibition (25 mm) against *Escherichia coli*. Wound healing studies using an excision wound model in rats showed significant improvement in wound contraction with the polyherbal gel compared to individual plant formulations and the control. The enhanced activity is attributed to the synergistic effect of phytoconstituents from both plants. These findings suggest that the *Euphorbia hirta* and *Bryophyllum pinnatum* combination gel may serve as a promising topical agent for wound management.

KEYWORDS: *Euphorbia hirta*, *Bryophyllum pinnatum*, polyherbal gel, phytochemical screening, antioxidant activity, antibacterial activity, wound healing.

INTRODUCTION

Wound healing is a complex physiological process involving inflammation, proliferation, and tissue remodeling to restore the structural and functional integrity of injured skin. Despite advances in modern medicine, wound management remains a significant challenge due to the limitations of synthetic drugs, such as high cost, side effects, and delayed healing. In recent years, there has been growing interest in the use of herbal medicines for wound care, owing to their safety, biocompatibility, and synergistic pharmacological effects. **(Alsaffar et al. 2021).**

Euphorbia hirta (family: Euphorbiaceae), commonly known as Asthma plant, is traditionally used for its antimicrobial, anti-inflammatory, and wound healing properties. Phytochemical studies have revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds that contribute to its therapeutic activity. Similarly, *Bryophyllum pinnatum* (family: Crassulaceae), also known as Patharchatta, is widely used in traditional systems of medicine for treating wounds, ulcers, and infections. It contains bioactive constituents such as flavonoids, glycosides, steroids, and phenols, which possess antioxidant and antimicrobial activities that promote tissue regeneration. **(Bigoniya et**

al. 2014; Papiya Bigoniya et al. 2014; Araújo et al. 2023; Gadge & Gadge 2025).

Polyherbal formulations, which combine multiple plant extracts, are increasingly recognized for producing enhanced therapeutic outcomes through synergistic interactions of their bioactive compounds. The development of a polyherbal gel containing *Euphorbia hirta* and *Bryophyllum pinnatum* offers a promising topical approach for wound healing by combining the pharmacological potential of both plants.

The present study focuses on the extraction, phytochemical evaluation, formulation, and characterization of herbal gels prepared from *Euphorbia hirta* and *Bryophyllum pinnatum* extracts. The prepared formulations were evaluated for their physicochemical properties, antibacterial activity, and wound healing potential using animal models to establish a scientific basis for their traditional use and future therapeutic application.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Leaves of *Euphorbia hirta* and *Bryophyllum pinnatum* were collected, shade-dried at room temperature, and stored in airtight glass containers. The plants were authenticated by a plant taxonomist, and voucher specimens were deposited (Authentication No. **201/Saif./Sci./Clg/Bpl**).

Extraction Procedure

Coarsely powdered leaves (300 g) of each plant were successively extracted using petroleum ether (defatting) and methanol in a Soxhlet apparatus for 36 h. The extracts were concentrated under reduced pressure using a rotary evaporator and stored in airtight containers. Extraction yield (%) was calculated using the formula:

Preliminary Phytochemical Screening

Methanolic extracts were subjected to standard qualitative tests for the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, glycosides, and phenolic compounds using established chemical methods. **(Tangco JVV et al. 2015).**

Quantitative Phytochemical Estimation

Total Phenolic Content (TPC): Determined using the Folin–Ciocalteu method. Absorbance was measured at 760 nm, and results were expressed as mg Gallic Acid Equivalent (GAE)/g dry extract. **(Parthasarathy S et al. 2009)**

Total Flavonoid Content (TFC): Estimated by the aluminium chloride method, with absorbance at 510 nm. Results were expressed as mg Rutin Equivalent (RE)/g dry extract.

In-Vitro Antioxidant Activity (DPPH Assay)

Methanolic solutions (20–100 µg/mL) of extracts and standard were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm. Radical scavenging activity (%) was calculated as:

% Inhibition = [(Ab of control- Ab of sample) / Ab of control x 100]

Acute Toxicity Study

Acute toxicity was evaluated as per OECD guidelines using the acute toxic class method. Test extracts were administered orally to female Wistar rats (n = 3 per step) at doses of 5, 50, 300, and 2000 mg/kg body weight to determine the safe dose range. **(OECD Environment, Health and Safety Publications; 1996)**

Formulation of Topical Gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and Formulations I, II, were 1% of each concentration of extract and formulation III was 2% concentration (i.e. 1% of each extract) were incorporated into the dispersion to obtained gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps**. (Lacman L, 1987).**

Table 1: Composition of prepared herbal gel.

Name of Ingredient	Formulation I	Formulation II	Formulation III
Carbopol 940	1 gm	1 gm	1 gm
Carboxymethyl cellulose	1 gm	1 gm	1 gm
Propylene glycol	0.5 ml	0.5 ml	0.5 ml
Methyl paraben	0.2 ml	0.2 ml	0.2 ml
Euphorbia hirta	1 gm	----	1 gm
Bryophyllum pinnatum	----	1 gm	1 gm
Triethanolamine	q.s	q.s	q.s
Water	100 ml	100 ml	100 ml

Characterization of Gel Formulations
The prepared gels were evaluated for appearance, pH, viscosity (Brookfield viscometer, spindle no. 61, 100 rpm, 25°C), and spreadability using standard procedures. **(Kumar TP et al. 2020; McGlynn W, 2003; Monica AS et al. 2014; Sandeep DS, 2020)**

Skin Irritation Test
The formulations were applied to the shaved dorsal skin of Wistar rats (150–200 g). Animals were observed for 24 h for erythema or edema to evaluate dermal irritation. **(Giri MA et al. 2019; Murthy SN et al. 2001)**

Wound Healing Study
A partial-thickness excision wound model was used in Wistar rats (CPCSEA Approval No. **1646/PO/Re/S/11/CCSEA**). Animals were divided into five groups (n=6): control, Formulation I, Formulation II, Formulation III, and standard (Gentamicin gel). Gels were applied topically once daily for 21 days. **(Barua CC et al. 2010; Ko J et al. 2005)** Wound contraction (%) was calculated as:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound area} - \text{Specific day wound area}}{\text{Initial wound area}} \times 100$$

Antimicrobial Activity
Antibacterial activity of gel formulations was determined by the agar well diffusion method against *E. coli*. Nutrient agar plates were inoculated with standardized bacterial suspension (10^8 CFU/mL). Wells were loaded with varying concentrations (25–100 µg/mL) of formulations and incubated at 37°C for 24 h. The zones of inhibition were measured in millimeters. **(Mohammadi-Sichani M et al. 2012)**

RESULTS AND DISCUSSION

Result of Percentage Yield
In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Euphorbia hirta* and *Bryophyllum pinnatum* is shown in Table: 2

Table 2: Percentage Yield of crude extracts of *E. hirta* and *B. pinnatum* extract.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	Euphorbia hirta	Pet ether	302	1.58	0.52%
2		Methanol	350	5.10	1.86%

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	Bryophyllum pinnatum	Pet ether	320	1.25	0.39%
2		Methanol	355	4.99	1.40%

Results of Preliminary Phytochemical study
Table 3: Phytochemical testing of *E. hirta*.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1	Alkaloids	-	+
2	Glycoside	-	+
3	Carbohydrates	+	-
4	Proteins and Amino Acids	-	-
5	Flavonoids	-	+
6	Tannin and Phenolic Compounds	-	+
7	Saponin	-	+
8	Triterpenoids and Steroids	-	+

Table 4: Phytochemical testing of B. pinnatum.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1	Alkaloids	-	-
2	Glycoside	-	+
3	Carbohydrates	-	-
4	Proteins and Amino Acids	-	+
5	Flavonoids	-	+
6	Tannin and Phenolic Compounds	-	+
7	Saponin	-	+
8	Triterpenoids and Steroids	-	+

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation

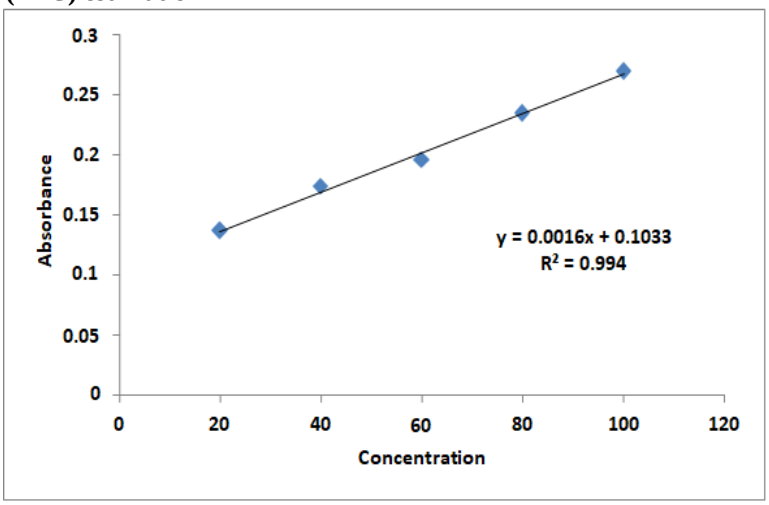


Figure 1: Graph represent standard curve of Gallic acid.

Table 5: Total Phenolic content in extract.

Total phenolic content (mg/gm equivalent to gallic acid)		
Extracts	***Euphorbia hirta***	***Bryophyllum pinnatum***
Absorbance (Mean±Sd)	0.189±0.07	0.201±0.09
TPC	86	98

Total Flavonoids content (TFC) estimation

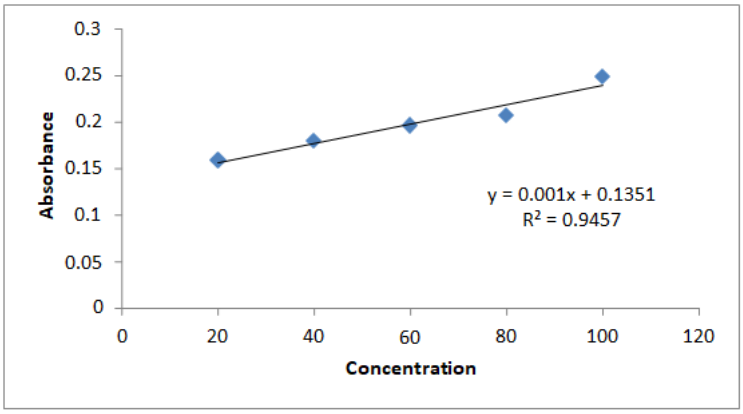


Figure 2: Graph represent standard curve of Rutin.

Table 6: Total Flavonoid Content in extracts.

	Total flavonoid content (mg/gm equivalent to rutin)	
Extracts	***Euphorbia hirta***	***Bryophyllum pinnatum***
Absorbance (Mean±Sd)	0.159±0.010	0.178±0.009
TFC	24	43

Assessment of Free Radical Scavenging Activity by DPPH

In the current study, the *in-vitro* anti-oxidant activity of *E. hirta* and *B. pinnatum* extracts was assessed using DPPH radical scavenging.

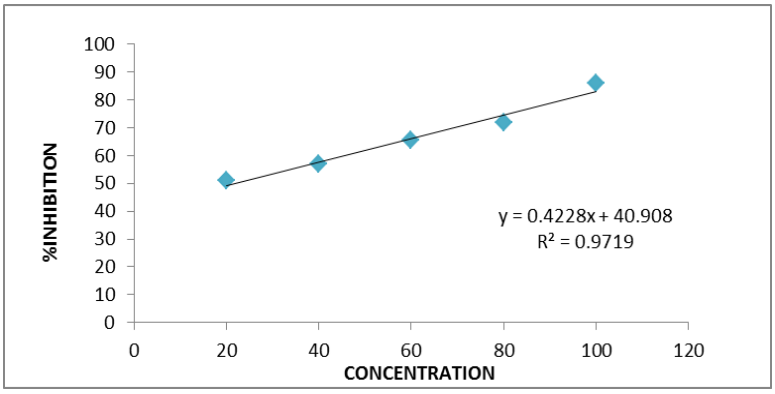


Figure 3: DPPH radical scavenging activity of Std. Ascorbic acid.

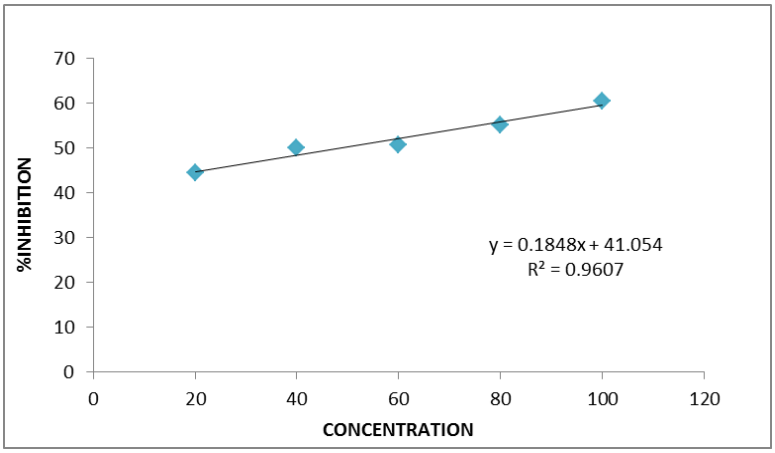


Figure 4: Represents the Percentage Inhibition Vs Concentration of extract of *E. hirta.*

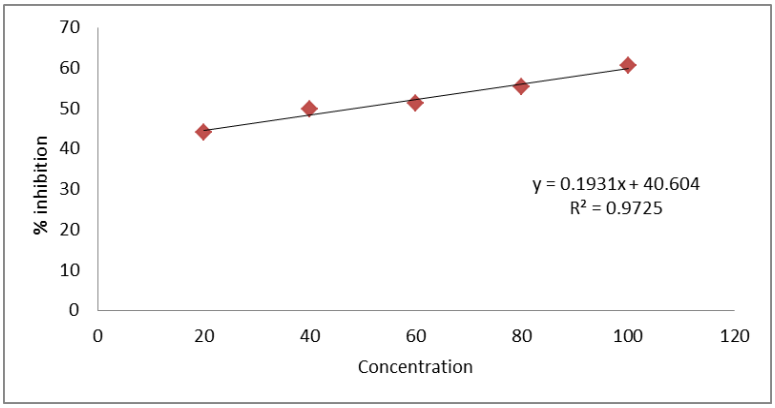


Figure 5: Represents the Percentage Inhibition Vs Concentration of extract of *B. pinnatum.*

Evaluation parameter of herbal gel formulation

Results of Organoleptic properties

Table 7: Organoleptic properties.

S. No	Parameters	Results
1.	Appearance	Semisolid gel
2.	Colour	Slightly yellowish grey gel
3.	Homogeneity	Absence of aggregates

An evaluation of the gel, including colour, appearance and homogeneity, was conducted. Gel was discovered to have a slightly yellowish grey colour to it when tested. Gel exhibited the same colour, and Appearance as the I.P. requirements for these characteristics and the results were listed in Table No.7.

Measurement of pH

Table 8: pH determination.

S. No	Formulation	Results
1.	Formulation1	6.3
2.	Formulation 2	5.9
3.	Formulation 3	6.6

The pH of all prepared formulation ranged from 5.9- 6.6. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. The results were shown in Table No.8.

Determination of Viscosity

Table 9: Viscosity determination.

S. No	Formulation	Results (cps)
1.	Formulation1	1454±0.71
2.	Formulation 2	1432±0.25
3.	Formulation 3	1487±0.86

Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from gel. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 7. the results were shown in Table no.9.

Determination of Spreadability

Table 10: Spreadability test.

S. No	Formulation	Results (gm.cm/sec)
1.	Formulation1	23.44
2.	Formulation 2	22.30
3.	Formulation 3	24.19

Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. Spreadability of different gel formulation was studied. The formulations produced good spreadability and the results were shown in Table no. 10

Results of Acute skin irritation study

Table 11: skin irritation study.

S. No	Formulation	Results
1.	Formulation1	Not irritant observed
2.	Formulation 2	Not irritant observed
3.	Formulation 3	Not irritant observed

Results of skin irritation test indicate that prepared gels were not produce irritation, redness, or edema on application and free from dermatological reaction.

Results of Wound contraction studies

Wound contraction is another parameter used to assess wound healing. Significant wound contraction was shown in table.

Table 12: Percentage wound closure in various treatment groups.

Sr. No.	Formulation	Area of wound during different days of observation (%)				
		4 day	8 day	12 day	16 day	21day
1	Control	8.31±0.716	8.48±0.815	8.44±0.781	8.39±0.885	8.42±0.982
		0	2	9	2	5
2	Formulation I	9.23±0.404	24.26±0.43	35.20±0.70	69.90±0.54	71.95±0.55
		2	50	87	17	22
3	Formulation II	8.23±0.782	18.19±0.52	50.10±0.55	71.90±0.55	74.29±0.58
		1	32	12	10	10
4	Formulation III	10.87±0.85	27.39±0.95	56.17±0.66	88.09±0.33	90.31±0.38
		95	20	77	58	28
5	Reference	11.89±0.74	31.03±0.72	60.00±0.64	89.99±0.64	92.55±0.25
	Standard (Gentami cin gel)	87	75	25	32	25

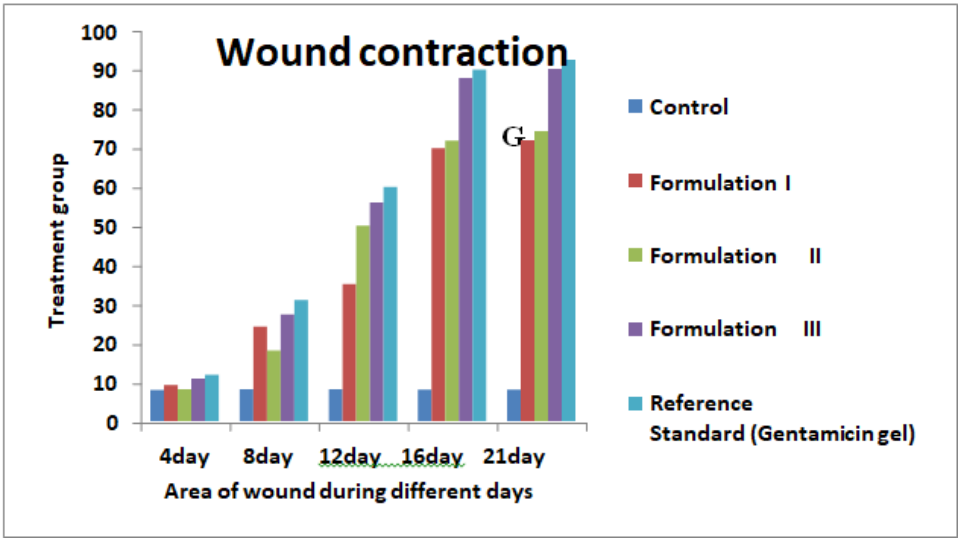
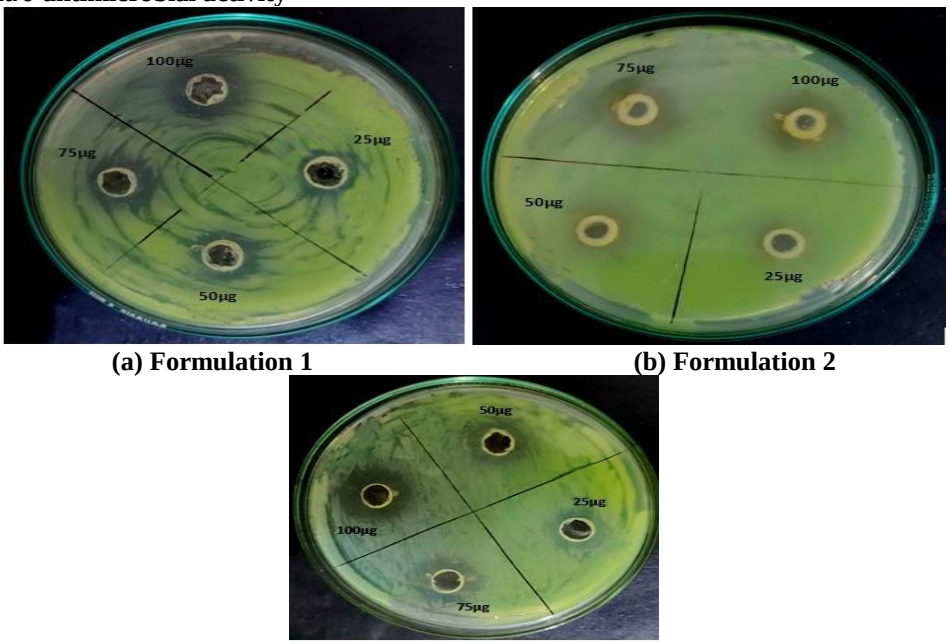


Figure 6: Wound Contraction.

Results of *In-vitro* antimicrobial activity



(a) Formulation 1 **(b) Formulation 2**

(c) Formulation 3

Figure 7: Antimicrobial Study (a) Formulation 1 (b) Formulation 2 (c) Formulation 3.

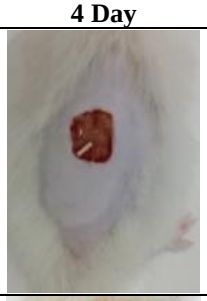
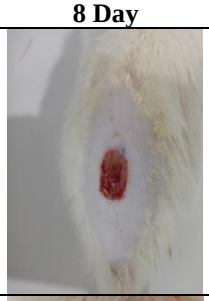
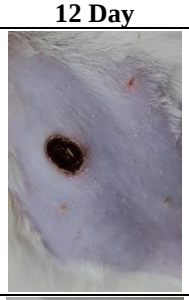
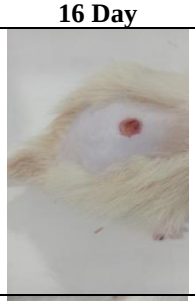
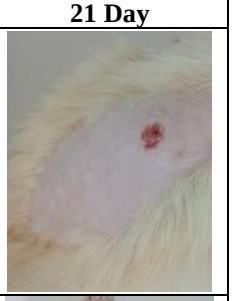
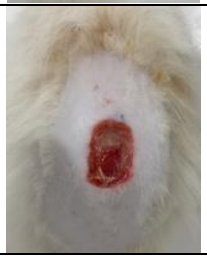
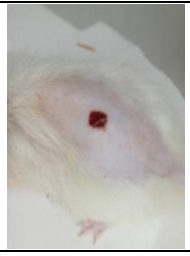
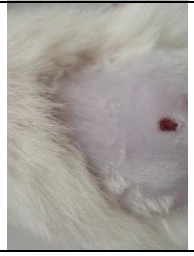
Table 13: Antimicrobial activity.

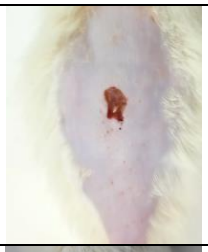
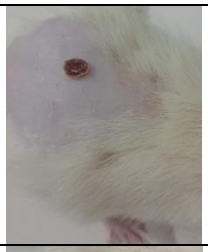
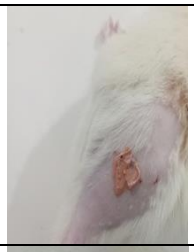
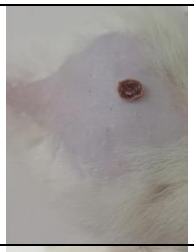
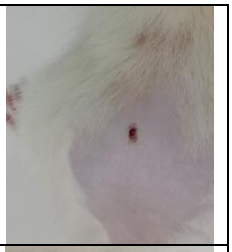
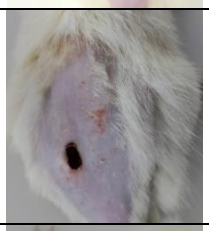
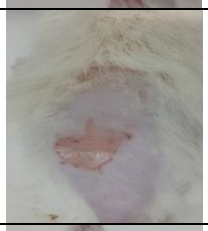
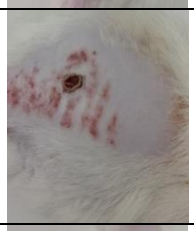
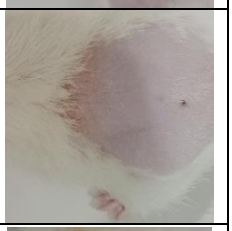
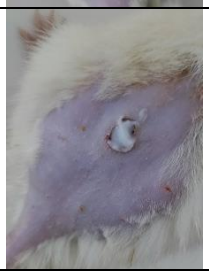
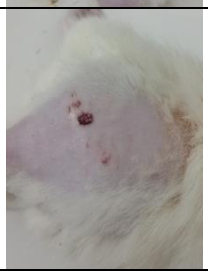
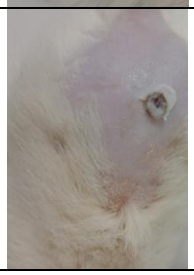
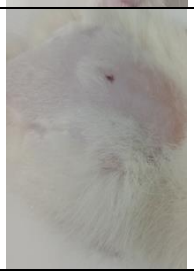
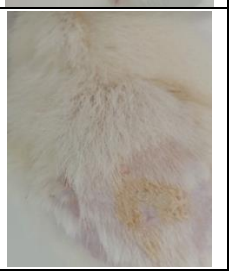
Concentration (µg/ml)	Zone of Inhibition (in mm)		
	Formulation 1	Formulation 2	Formulation 3
25	10mm	8 mm	12mm
50	13 mm	11 mm	14 mm
75	15 mm	14 mm	16 mm
100	17 mm	18 mm	20 mm

The in vitro antibacterial activities of the extracts of Formulation 1, Formulation 2 and Formulation 3 samples have been investigated. Antibacterial activity was performed against E. coli by well diffusion assay with concentration ranging 25, 50, 75 and 100µg/ml.

Formulation 1 extract showed best zones of inhibition of 20 mm in diameters at 100µg/ml concentration against E. coli. Similarly, Formulation 2 extract showed best zones of inhibition of 19 mm in diameters at 100 µg/ml concentrations against E. coli. Formulation 3 showed best zones of inhibition of 25 mm in diameters at 100µg/ml concentration against E. coli.

Table 14: Images of wound closure in various treatment groups.

Group	4 Day	8 Day	12 Day	16 Day	21 Day
Control					
Formulation I					

Formulation II					
Formulation III					
Standard(Gentamicin gel)					

CONCLUSION

The developed polyherbal gel containing *Euphorbia hirta* and *Bryophyllum pinnatum* extracts demonstrated superior wound healing activity compared to individual formulations. The enhanced effect may be attributed to the synergistic interaction of bioactive constituents such as flavonoids, phenolics, and saponins present in both plants. The formulation exhibited good physicochemical stability, was non-irritant to the skin, and showed significant antibacterial activity against *E. coli*. Overall, the study highlights the potential of the polyherbal gel as an effective and safe topical formulation for wound healing, providing a foundation for future pharmacological and clinical investigations.

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